The Effect of Organic Vapors on the Stability of Aerosols

By Yoshiyasu FUJITANI

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It is a well-known fact that aerosols are unstable by nature. The particles decrease in number rapidly both by coagulation due to mutual collision and by sedimentation under the gravity. Therefore, it would be difficult to define the stability of an aerosol system by a mathematical expression, since it is affected by many factors which may be co-operative. In evaluating the stability of an aerosol system, the rate of decrease in i) the particle number concentration[1], or ii) the mass concentration[2] is mainly measured and, in some cases, iii) the decrease in optical density or iv) the sedimentation velocity of boundary surface of the system[3] is also observed. The method i) is most essential, but requires skillful techniques and elaborate operation; whilst, the methods ii) to iv) are applicable merely to a routine work.

The coagulation processes of aerosol particles obey Smoluchowski's theory concerning the relationship between the time elapsed and the number of particles of colloidal dispersions. In spite of the theory being based on the many assumptions, the equation has been widely used so it is convenient to adopt his coagulation constant as a measure of the stability of an aerosol system. However, few investigations along these lines are available though numeous reports have been published regarding the stability of aerosols.

It may be expected that, when a substance which exhibits a capillary activity is added to an aerosol, the rate of coagulation of the particles may be influenced both by the nature and the concentration of the admixture. If this is the case, it may offer a most interesting problem on the collision process of aerosol particles, since it has been generally believed that the collision efficiency of aerosol particles is equal to unity under normal circumstances[4].

In a previous paper the stabilizing effect of the vapors of such organic surfactants as lower series of mono-functional alcohols (C_1 to C_5), acetone and ethyl acetate has been established for the fog of aqueous ammonium chloride solution, but the detailed mechanism of the coagulation process delayed by these vapors has not yet been made clear[5].

In the present paper, to compare the stabilities of aerosols depending on the nature of the added vapors as on well as their concentrations and to inquire into the role of the vapors in stabilizing the aerosols, the rate of coagulation in the presence of such vapors was observed by means of the ultramicroscopic method, using three kinds of aerosol, i.e., the fogs of aqueous ammonium chloride solution, glycerol and dioctyl phthalate (D.O.P.).

Experimental

The apparatus used for the present work consists of the following parts—an air-driving train, an aerosol generator, a vaporizer for the admixture, a smoke chamber and an ultramicroscope. The functions of these parts are mentioned successively.

The aerosols were prepared by two methods.

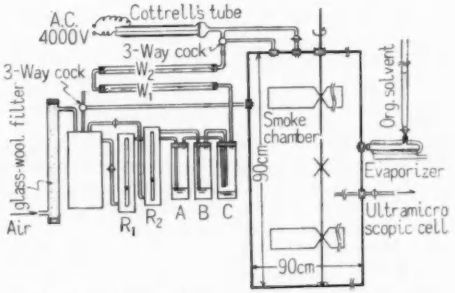

Fig. 1. Preparative system of the fog of aqueous NH_4Cl solution and other constructions.

1) R. Whytlaw-Gray and H. S. Patterson, "Smoke", Edward Arnold and Co., London, 1932, Chapter 5 and 6.
2) Y. Fujitani, *J. Chem. Soc. Japan, Pure Chem. Section*, **74**, 661 (1953).
3) Ibid., **73**, 817 (1952); **75** 89 (1954).
4) Ref. 1., P. 184.; "Handbook on aerosols", The Uuited Stated Atomic Energy Commission, Washington, DC., 1950, p. 65.
5) Y. Fujitani, *J. Chem. Soc. Japan, Pure Chem. Section*, **73**, 817 (1952), **74**, 704 (1953).

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a) **The fog of aqueous ammonium chloride solution.**—The fog of aqueous ammonium chloride solution was obtained by almost the same way as used in the previous experiments[5], and its modified generating system is indicated in Fig. 1. In this figure, A and B are 9.6 N hydrochloric acid and 11.2 N aqueous ammonia washing bottles, respectively, each containing 100 c.c. of the reagent, and bubbling velocity of clear air coming from a compressor through a glass wool filter and being regulated by the rota-meters, R_1 and R_2, is 0.7 l./min. for the former, and 0.3 l./min. for the latter, respectively. The gases of hydrogen chloride and ammonia driven from these bottles were mixed in the reaction chamber, C, where ammonium chloride smoke was produced. During the passage of the ammonium chloride smoke over a water surface in two glass tubes, W_1 and W_2, (each 2.0 cm. in inside diameter and 60 cm. long), ammonium chloride particles were changed to the fog droplets of aqueous ammonium chloride solution because of a strong tendency of ammonium chloride particle to absorb water vapor[6]. At the same time, the excess gas, chiefly ammonia gas, is absorbed through the water surface. Therefore, water in these two tubes, W_1 and W_2, was renewed in each run for obtaining a good reproducibility in fog preparation.

The concentration of ammonium chloride in the fog particle was found to be about 20 % by weight as an average which was determined by collecting the droplets of fog through a Cottrell's precipitator and analyzing them chemically by means of Mohr's method, a maximum deviation being less than 3 % by weight. Table I shows the mass

TABLE I

AVERAGE RADII AND MASS CONCENTRATIONS OF THE FOG OF AQUEOUS NH_4Cl SOLUTION OBSERVED UNDER THE SAME EXPERIMENTAL CONDITIONS

No. of expt.	Average radii (μ)	No. of expt.	Mass concentration (mg./l.)
1	0.24	2	17.9
3	0.20	4	18.1
5	0.24	6	18.3
7	0.23	8	17.9
9	0.21	10	17.4
Mean	0.22	Mean	17.9

concentrations and average radii of aqueous ammonium chloride droplets. Average radii shown in the table were determined from the droplet sizedistribution curve obtained by measuring the settling velocity of each fog droplet in an ultramicroscopic cell. The number of fog droplets observed is about 300 for one size-distribution curve. The size-distribution curve is depicted in Fig. 2. It can be seen from Fig. 2 and Table I that the reproducibility is very good.

b) **The fogs of glycerol and D. O. P.**—The fogs of glycerol and D.O.P. were prepared by an all-glassed L-type generator[7], which was the

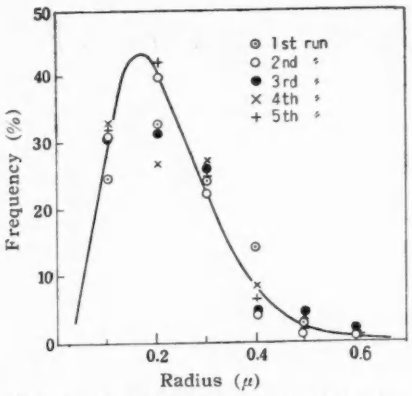

Fig. 2. Size-distribution curve of the fog of aqueous NH_4Cl solution obtained by the measurement of settling velocity of droplets.

modified LaMer-Sinclair's monodisperse aerosol generator[8], consisting of three parts—boiler, reheater and condensation tube. The generator is illustrated in Fig. 3. The boiler (horizontal

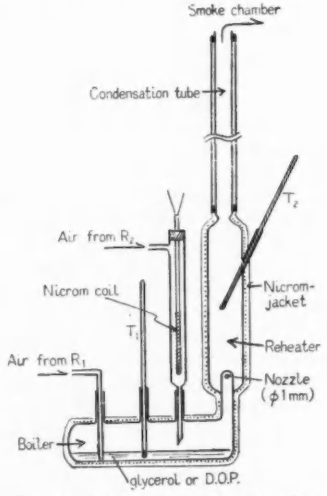

Fig. 3. L-type aerosol generator.

part, 3.5 cm. inside diameter, 20 cm. long) in which liquids or solids to be dispersed were placed, and the reheater (vertical part, 3.5 cm. inside diameter, 18 cm. long) were both electrically heated at the desired temperatures with a nicrom-jacket through variable voltstats. These two temperatures were measured by thermometers, T_1 and T_2, respectively. The condensation tube was a double-wall glass tube, and its inner tube is 2.0 cm. in inside diameter and 25 cm. long.

6) S. Twomey, *J. Applied Phys.*, **24**, 1099 (1953).
7) To be published for detailed description.
8) V. K. LaMer and D. Sinclair, *Chem. Rev.*, **44**, 245 (1949).

In order to obtain a good monodisperse fog by this generator, the temperatures must be kept properly for each material and the condensation nuclei added in a proper amount. Nuclei were supplied by an electrically heated nicrom-wire which was coiled along a quartz tube 8 cm. long.

The amount of glycerol or D.O.P. put in the boiler is 30 c.c.. The vapor driven from the boiler by bubbling air and the air laden nuclei were completely mixed in the reheater through two nozzles of 1 mm. diameter. The flow velocities of vapor and nuclei are both 0.5 l./min.. The mixture of vapor and nuclei was passed through a condensation tube where slow cooling and subsequent condensation of vapor on nuclei occurred.

The fogs produced were so nearly monodisperse that higher order Tyndall spectra[8] were visible. The temperatures both in the boiler and the reheater under the above mentioned preparative procedure and the average radii obtained under these conditions are as shown in Table II for

TABLE II
TEMPERATURES OF PREPARATIVE CONDITIONS AND AVERAGE RADIUS OF DROPLET

Substances	Temperature of generator (°C)		Average radius (μ)
	Boiler	Reheater	
Glycerol	135	145	0.18
D.O.P.	160	180	0.22

glycerol and D.O.P.. Average radii were determined from the measurements of settling velocity in the same manner as mentioned above.

The fogs produced by these procedures, a) and b), were re-dispersed homogeneously by stirring for 5 min. in a smoke chamber which is a wooden octagonal box coated with paraffin, 90 cm. in height, its content being 700 l.. The initial particle concentration in the smoke chamber was maintained at 2 to 5×10^5 per c.c. through out the whole experiment.

Organic liquid to be tested for its stabilizing action was evaporated into the smoke chamber from a vaporizer which was made of a quartz tube, 2.0 cm. in inside diameter and 20 cm. long, having a side tube connected to a burette. The organic liquid was poured into the vaporizer from the burette. The vaporizer was electrically heated by a nicrom-jacket. The concentration of organic vapor in the smoke chamber, expressed in m. mol./l., was calculated from the burette reading. The mixture of fog and organic vapor was stirred homogeneously for 5 min. as in the case of the fog alone.

The organic liquids used were carefully purified by the usual procedure and their boiling points were found to coincide fairly well with those of the literature[9],

The number of particles per unit volume is counted by an ultramicroscopic technique. The volume of visible field in the ultramicroscopic cell was 1×10^{-6} c.c.. The brilliant points appearing in the dark field were counted and summed up for a fog droplet driven successively into the ultramicroscopic cell. The counting of fog droplets is done every 3 to 5 min. for an interval of 60 to 120 min.. The magnification of ultramicroscope was ×80.

All experimentals were carried out at room temperature, 16 to 19°C..

Results

The decrease in number of fog droplets determined ultramicroscopically is shown in Fig. 4, 5 and 6 as the typical examples.

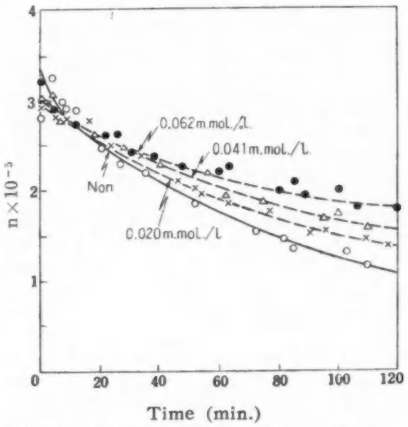

Fig. 4. Variation of droplet number per c.c. as a function of time and concentration of ethyl acetate mixed to the fog of aqueous NH_4Cl solution.

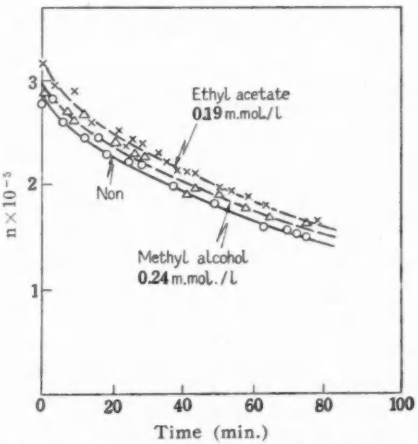

Fig. 5. Variation of droplet number per c.c. as a function of time and some vapors mixed to the fog of glycerol.

9) A. Weissberger et. al., "Organic Solvents···Physical Properties and Methods of Purification", 1955.

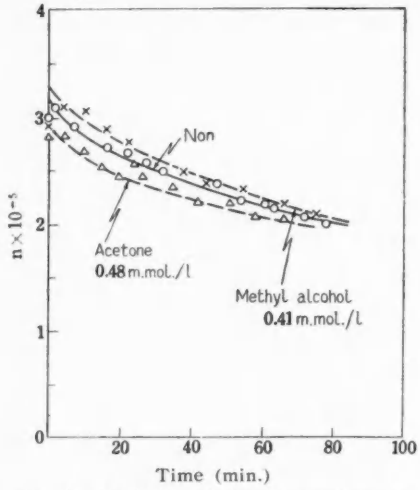

Fig. 6. Variation of droplet number per c. c. as a function of time and some vapors mixed to the fog of D.O.P..

In these figures, solid curves represent a decrease in number of fog droplets for a fog system without organic vapor, and broken curves refer to one with organic vapor. All curves showed a tendency to decrease monotonously in the course of aging.

Converting these droplet number—time graphs into a reciprocal droplet number—time ones, the linear relationships are

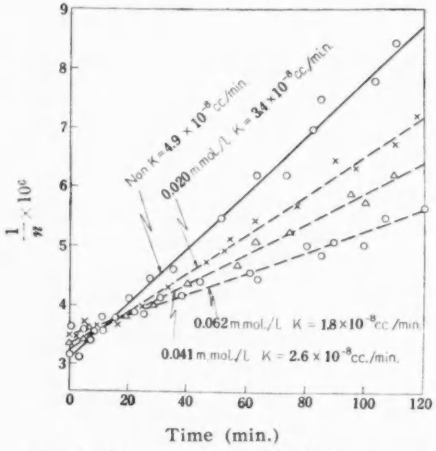

Fig. 7. Linear relationship between reciprocal number of droplet and time obtained as an influence of ethyl acetate concentration mixed to the fog of NH_4Cl-aqueous solution. K is a coagulation constant calculated from the slop of observed line.

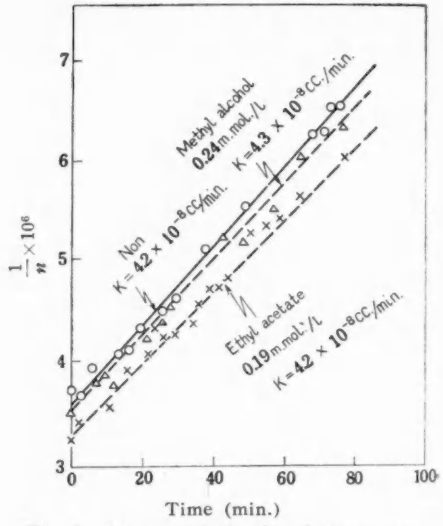

Fig. 8. Linear relationship between reciprocal number of droplet and time obtained as an influence of some vapor mixed to the fog of glycerol.

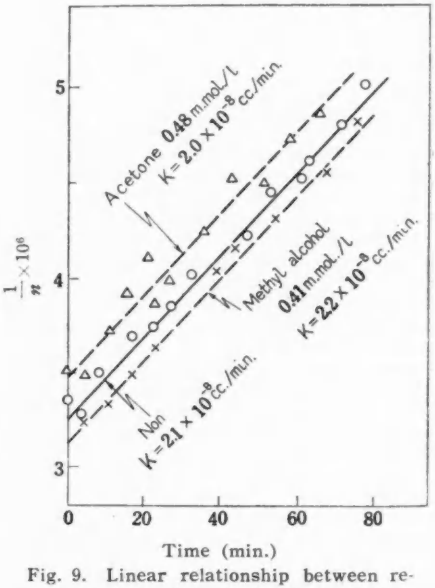

Fig. 9. Linear relationship between reciprocal number of droplet and time obtained as an influence of some vapors mixed to the fog of D.O.P..

obtained for all of them as in Figs. 7, 8 and 9. The linear relationship holds throughout all the runs of experiment. It may be noticed readily that the slope of line varies markedly with the nature

and the concentration of the vapor added to the fog of aqueous ammonium chloride solution, while, in contrast with this, it remains almost unaltered in the case of the fogs of glycerol and D.O.P. even in a greater concentration.

Smoluchowski's equation is expressed as follows,

$$1/n-1/n_0=Kt, \quad (1)$$

or in a more correct form,

$$1/n-1/n_0=K'(1+A\lambda/r)t \quad (2)$$

where n_0 and n are the numbers of droplets per unit volume at the begining, $t=t$, and time t, respectively, K and K' are the coagulation constants equal to $4kT/3\eta$ (k: Boltzman's constant, η: viscosity of air) numerical value being 1.8×10^{-8} c.c./min. in air at $T=293°$K, λ the mean free path of air molecules, r the radius of fog droplet, and A a constant having a value of 0.7 to 1.7. $A\lambda/r$ in equation (2) gives a correction term arising from a slipage between a fog droplet and air molecules during the movement of a fog droplet in still air; therefore, the smaller the value r becomes, the greater becomes the value $A\lambda/r$.

Thus, the slopes obtained experimentally are equal to K. However, K might be used instead of K', for the comparison of relative stabilizing effects of organic vapors on the same aerosol since the droplet size-distribution curve was practically unchanged for the fogs used in all runs.

The dependence of coagulation constant, K or K', upon the concentration of various organic vapors mixed are plotted in Figs. 10 and 11 for the fogs of aqueous ammonium chloride solution, glycerol and D.O.P..

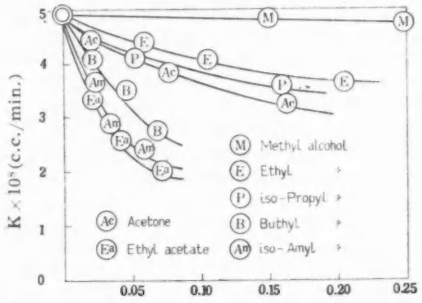

Fig. 10. Variation of the coagulation constant, K, with the concentration of mixed vapors for the aqueous NH_4Cl solution fog. Symbol ◎ is the average coagulation constant for a pure fog and its value is 4.9×10^{-8} c.c./min..

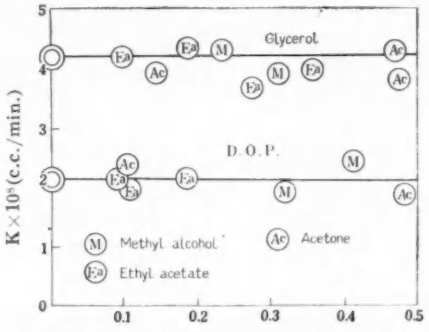

Fig. 11. Variation of the coagulation constant as a concentration of mixed vapors for the fogs of glycerol and D.O.P.. Symbols ◎ are the coagulation constant for the pure fog of glycerol and D.O.P. and their values are 4.2×10^{-8}c.c./min. and 2.1×10^{-8}c.c./min., respectively.

From these figures, it is found that for the same vapor the more it is added, the more strongly the fog of aqueous ammonium chloride solution is stabilized, but no effect is observed for the other fogs within the limit of experimental precision. For the former, the coagulation constant falls off to nearly one-third under the most stabilized condition.

Discussion

Although a number of studies have been made by Russian workers and others since 1930 concerning the effects of admixture[10], the information obtained is not always in agreement with one another, because the experimental conditions and procedures were not unified. Especially, the defect of a criterion for the ability of stabilization must be noticed.

The stability of aerosol should be governed by the internal stability[11], expressed in terms of the particle concentration and the particle size-distribution, both of which are determined a priori by the condition of formation, provided that the collision efficiency has a constant value in all of the collision processes. Therefore if the collision efficiency of aerosol particles may be altered as suggested by Patterson[12] either by the

10) T. Tatibana, *J. Chem. Soc. Japan, Pure Chem. Sec.* **68**, 53 (1947); I. S. Artemov, *J. Phys. Chem., (U. S. S. R.)*, **20**, 533 (1946); I. V. Petryanov et al., ibid., **15**, 811 (1941); etc.

11) D. Sinclair, "Air Pollution", Chapter 18, p. 169, McGrow-Hill Book Co., 1952.

12) Ref. 1., Chapter 16.

TABLE

EFFECT OF VAPORS ON THE INCREASE OF CHARGED

Fog	Sign of elec. charge	Vapor concn. (m.mol./l.)	Percentage of charged droplet for various time in the aging time (min.)					
			0	10	20	30	40	60
Aqueous NH_4Cl solution	+	Non	9.1	9.9	14.0	14.2	15.5	19.6
	−		8.8	11.0	11.3	16.0	14.9	19.2
Glycerol	+	Non	6.5	7.9	9.5	14.5	12.8	18.8
	−		8.1	12.2	13.2	15.4	22.1	27.0
D.O.P.	+	Non	14.0	17.1	19.6	27.0	28.2	37.3
	−		13.7	14.8	17.3	20.2	23.9	27.6

physico-chemical structure of surface or by the changes in surface state, we may have a most important problem which has been disregarded up to date. This will be developed from now on. To study this in an orthodox way, it is better to evaluate the coagulation constants experimentally for various fogs to which vapors are added, and to compare them with one another.

From this point of view, the experimental results obtained above are interesting by reason of a large difference among the values of the coagulation constant for the fog of aqueous ammonium chloride solution and the degree of lowering of these values which are parallel with a concentration of mixed vapors. The tendency of a stabilizing effect of mixed vapor agrees with the Traube's rule[13] referring to the surface activity of the homologous series of organic compounds on pure water surface. These facts are considered to be valuable as the fundamental basis in discussing the stabilities of aerosols. It may be inferred that the coalescence of droplets in the collision process is obstructed by a decrease in surface tension somewhat in an analogous manner as the stabilization of emulsion system pointed out by Donnan[14]. Because these organic vapors have not shown capillary activity when mixed with the fogs of glycerol and D.O.P..

In a strict sense of the discussion concerning the effects of admixtures, it appears that many other factors should be taken into account with equal weight. These may be classified chiefly into four factors i.e., i)the changes in density and viscosity of medium, ii) the growth of droplets by the condensation of admixtures[15], iii) the change in distribution in electric charge of fog droplets, iv), particularly, in the case of the aqueous ammonium chloride solution, the disappearance of fog droplets by evaporation of water, etc..

First, the effects of i) and ii) have a relation to the mobility of fog droplets by the Brownian movement and sedimentation under the gravity during standing in a still medium. However, these influences caused by the change of physicochemical properties of the medium may be small.

Second, the growth of a fog droplet by the condensation of vapor may depend upon its partition ratio between the vapor and the droplet phase, but the concentration of vapor in all experimental conditions is too low to make a droplet grow larger. In fact, no appreciable changes of fog droplet size were ascertained by ultramicroscopic observation.

Third, the change in the proportion of a charged droplet was similar irrespective of the presence of vapor during the aging. The results obtained from the electrophoresis of charged droplets in an electric field of an ultramicroscopic cell are shown in Table III. The contribution of an electric charge on the coalescence process has been also proved to be small[16].

Finally, it is generally accepted that a dissipation of aqueous aerosol by evaporation is very rapid, but, in the present case, the fog droplets consist of an aqueous ammonium chloride solution which is so concentrated that the fog system is very stable.

If the contribution of these factors is not small, it must result in a change in the coagulation constant for the fogs of glycerol and D.O.P.. Therefore, these effects are all negligible in the present work as clearly seen from the afore-said experimental results.

Most of the discussion has gone no

13) I. Traube, *Ann.* **265**, 27 (1891).
14) Donnan, *Z. physik. Chem.*, **31**, 42 (1899).
15) V. K. LaMer and R. Gruen, *Trans. Faraday Soc.*, **48**, 410 (1952); V. K. LaMer and S. Coston, *Science*, **118**, 516 (1953).
16) W. B. Kunkel, *J. Applied Phys.*, **19**, 1053 (1948).

III

DROPLET PERCENTAGE DURING THE AGING

Increase rate of charged droplet (%/min.)	Vapor concn. (m.mol./l.)	Percentage of charged droplet for various times aging time (min.) 0	10	20	30	40	60	Increase rate of charged droplet (%/min.)
0.17	0.24 Acetone	9.9	10.3	13.5	16.2	16.2	20.1	0.17
0.17		9.4	12.7	14.4	15.0	17.1	21.9	0.19
0.22	0.42 Acetone	6.1	9.4	11.2	10.2	16.1	19.1	0.20
0.31		9.0	10.2	15.3	18.4	19.5	27.3	0.32
0.37	0.39 Ethyl	15.1	20.0	23.4	25.0	28.3	39.5	0.37
0.23	acetate	11.2	14.6	18.4	17.3	23.2	27.5	0.20

further than establishing a few general quantitative remarks, but there is a reason to believe that the mechanism of stabilizing action of the organic vapors on the fog system is largely attributable to a capillarity of admixtures. In order to prove that this conclusion is of general applicability, we need further studies, both theoretical and experimental, to be pursued as the more fundamental problems.

I wish to express my thanks to Prof. Isamu Sano of Nagoya University for his helpful advice.

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The Synthesis of 4′,5,7-Trihydroxy-8-methoxyisoflavone

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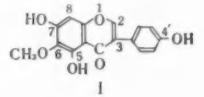
Tectorigenin[1] was isolated from *Iris tectorum Maxim* and the structure[2] of 4′, 5,7-trihydroxy-6-methoxyisoflavone (I) was assigned to it, but it is not yet synthesized. We have been attempting the syntheses of this isoflavone and related compounds. The present paper describes the synthesis of 4′,5,7-trihydroxy-8-methoxyisoflavone (II), an isomer of tectorigenin. Although it was shown by W. Baker et al.[3] that 4′,5,7-trihydroxy-8-methoxyisoflavone (II) was obtained from 2,4,6-trihydroxy-3-methoxyphenyl 4-hydroxybenzyl ketone (IV) with the ethoxalylation process[4], their communication did not give any detailed description of the procedure or of the melting points of the compounds. It appeared of interest to investigate further W. Baker et al's method in detail for the following reason. If the direction of ring closure of the ketone (IV) was opposite to that observed by them, the possibility of the synthesis of tectorigenin might occur. So it seemed desirable to prepare 4′,5,7-trihydroxy-8-methoxy-isoflavone (II) by an unambiguous synthetic method for comparison with the isoflavone prepared with the ethoxalylation process. Specimens of the isoflavone prepared by two alternative methods (see Experimental) proved to be the same compound (II). The experiments described here established, therefore, the fact that 4′,5,7-trihydroxy-8-methoxyisoflavone (II) was obtained with the methoxalylation and the ethoxalylation process, the direction of ring closure being the same as that observed by W. Baker et al.[3].



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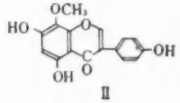
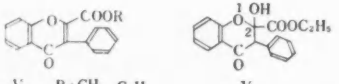


II

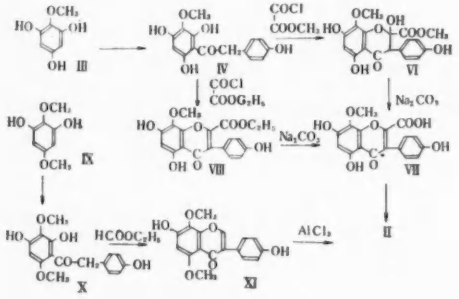
1) B. Shibata, *J. Pharm, Soc. Japan.* **47**, 380 (1927).
2) Y. Asahina, B. Shibata and *Z.* Ogawa, ibid., **48**, 1087 (1928).
3) W. Baker I. Dunstan, J. B. Harborne, W. D. Ollis and R. Winter, *Chem. and Ind.*, **1953**, 277.
4) W. Baker, J. Chadderton, J. B. Harborne and W. D. Ollis, *J. Chem. Soc.*, **1953**, 1852.

Iretol (III) when condensed with *p*-hydroxyphenylacetonitrile, gave 2, 4, 6-trihydroxy-3-methoxyphenyl 4-hydroxybenzyl ketone (IV), m. p. 220—1.5°. The ketone (IV) was treated at 0° with methoxalyl chloride in pyridine to give yellow crystals, m. p. 215—6.5°, which contained one molecule of water in excess of that required by the corresponding methyl isoflavone-2-carboxylate derivative (Va; R=CH_3). The cyclization of *o*-hydroxyphenyl benzyl ketones to isoflavone-2-carboxylic esters (Va; R=C_2H_5) with the ethoxalylation process was postulated as proceeding through the intermediates of type (Vb) which were isolated in some cases[4]. On the basis of this fact, it seemed reasonable to assume the structure of the yellow crystals to be methyl 2-hydroxyisoflavanone-2-carboxylate derivative (VI). This intermediate was converted into the corresponding isoflavone-2-carboxylic acid, m. p. 288—9° (VII) by mild alkaline hydrolysis and thence the acid was decarboxylated with copper powder in boiling quinoline to the isoflavone (II), m. p. 241—2°, in colorless needles, (its acetate, m. p. 167°). When the cyclization reaction was carried out with ethoxalyl chloride instead of methoxalyl chloride, the usual isoflavone-2-carboxylic ester, m. p. 219—20° (VIII), was obtained and it was converted into the acid (VII) by mild alkaline hydrolysis.



Va R: CH_3, C_2H_5 Vb

2, 5-Dimethoxyresorcinol (IX) was condensed with *p*-hydroxyphenylacetonitrile by Hoesch reaction to give 2, 4-dihydroxy-3, 6-dimethoxyphenyl 4-hydroxybenzyl ketone (X), m. p. 98—9.5°. This ketone (X) was converted into 4′, 7-dihydroxy-5, 8-dimethoxyisoflavone (XI) with ethyl formate in the presence of sodium. The action of aluminium chloride on XI in nitrobenzene caused partial demethylation (in the 5-position) without affecting the other methoxy group, and the demethylated product isolated was 4′, 5, 7-trihydroxy-8-methoxyisoflavone, m. p. 238—9° (II). When this was mixed with the isoflavone which had been prepared with the methoxalylation and the ethoxalylation process, no melting point depression occured.



Experimental*

2, 4, 6-Trihydroxy-3-methoxyphenyl 4-hydroxybenzyl ketone (IV).—A solution of iretol (4.1 g.)[5] and *p*-hydroxyphenylacetonitrile (4.5 g.) in absolute ether (100 ml.) containing anhydrous zinc chloride (2 g.) was saturated at 0° with dry hydrogen chloride. After being kept over-night, the viscous oily layer was separated, washed twice with ether and hydrolyzed with water (150 ml.) on a steam-bath for an hour. The solid which was separated on cooling was purified from aqueous methanol to form the ketone (4.8 g.) in colorless needles. m. p. 220—1.5°, having a violet ferric reaction in alcohol.

Anal. Found: C, 62.43; H, 4.99. Calcd. for $C_{15}H_{14}O_6$: C, 62.07; H, 4.83%.

Methyl 2, 4′, 5, 7-tetrahydroxy-8-methoxyisoflavanone-2-carboxylate (VI).—To a solution of 2, 4, 6-trihydroxy-3-methoxyphenyl 4-hydroxybenzyl ketone (2.4 g.) in pyridine (25 ml.), methoxalyl chloride (5.5 g.) was added drop by drop with stirring at 0°. After standing overnight, the mixture was poured into ice-water and extracted with chloroform. The chloroform solution was washed with dilute hydrochloric acid, dried over anhydrous sodium sulfate and evaporated. A part (0.3 g.) of the residue thus obtained was recrystallized from methanol. Yellow microcrystals, m. p. 215.5-6.5° were obtained.

Anal. Found: C, 57.49; H, 4.55. Calcd. for $C_{18}H_{16}O_9$: C, 57.45; H, 4.26%.

Alcoholic ferric chloride showed green color.

4′, 5, 7-Trihydroxy-8-methoxyisoflavone-2-carboxylic acid (VII).—To a solution of the remaining crude ester (VI) in acetone (30 ml.), 3% aqueous sodium carbonate solution (30 ml.) was added, and the mixture was refluxed for four hours. Acetone being evaporated on a steam-bath, the solution was cooled by ice-water and filtered. The product was isolated by acidification of the filtrate with dilute hydrochloric acid. Upon purification from aqueous methanol it gave rise to 4′, 5, 7-trihydroxy-8-methoxyisoflavone-2-carboxylic acid (1.1 g.) in orange yellow microcrystals, m. p. 288—9° (dec.), having a green ferric reaction in alcohol.

Anal. Found: C, 58.72; H, 3.82. Calcd. for $C_{17}H_{12}O_8$: C, 59.31; H, 3.51%.

* The melting points were uncorrected.

5) This was prepared from trinitroanisole by the analogous method to that used in the preparation of phloroglucinol. (H. T. Clarke and W. W. Hartman, *Organic Syntheses*, John Wiley and Sons, Inc., New York, 1932, Coll. Vol. I, p. 496).

Ethyl 4',5,7-trihydroxy-8-methoxyisoflavone-2-carboxylate (VIII) and the acid (VII)—Cyclization of 2,4,6-trihydroxy-3-methoxyphenyl 4-hydroxybenzyl ketone (1.7 g.) with ethoxalyl chloride (4.5 g.) in pyridine (25 ml.) in a similar way to that described before, followed by extraction with chloroform, gave rise to microcrystals of ethyl 4',5,7-trihydroxy-8-methoxyisoflavone-2-carboxylate, m. p. 219—20°.

Anal. Found: C, 61.22; H, 4.49. Calcd. for $C_{19}H_{16}O_8$: C, 61.29; H, 4.33%.

Hydrolysis of this ester in boiling acetone containing aqueous sodium carbonate solution gave rise to the free acid (0.8 g.) identical with the above sample.

2,4-Dihydroxy-3,6-dimethoxyphenyl 4-hydroxybenzyl ketone (X).—A solution of 2,5-dimethoxyresorcinol[6] (1.5 g.) and *p*-hydroxyphenylacetonitrile (1.2 g.) in absolute ether (50 ml.) containing anhydrous zinc chloride (0.5 g.) was saturated with hydrogen chloride at 0°. Isolation of the product in the usual way gave rise to the ketone (1.3 g.) which was purified from aqueous methanol in colorless needles, m.p. 98—9.5°, having a violet ferric reaction in alcohol.

Anal. Found: C, 62.70; H, 5.43. Calcd. for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30%.

4',7-Dihydroxy-5,8-dimethoxyisoflavone (XI).—A solution of 2,4-dihydroxy-3,6-dimethoxyphenyl 4-hydroxybenzyl ketone (1 g.) in ethyl formate (20 ml.) was added drop by drop with stirring to a suspension of powdered sodium (1 g.) in ethyl formate (10 ml.) at −4° under nitrogen. The reaction mixture was allowed to stand overnight at 10°, and was decomposed by the addition of ice-water. Ethyl formate being removed in vacuo, the residual aqueous solution was acidified and warmed on a steam-bath. The precipitate was collected and recrystallized from methanol to 4',7-dihydroxy-5,8-dimethoxyisoflavone (0.2 g.) in colorless needles, m. p. 274—5°, having a negative ferric reaction in alcohol.

Anal. Found: C, 64.90; H, 4.65. Calcd. for $C_{17}H_{14}O_6$: C, 64.96; H, 4.49%.

4',5,7-Trihydroxy-8-methoxyisoflavone (II). Method (a).—A mixture of 4',5,7-trihydroxy-8-methoxyisoflavone-2-carboxylic acid (0.6 g.) and copper powder (0.6 g.) in quinoline (30 ml.) was heated with stirring under nitrogen for ten minutes at 230—40° (bath temperature). After cooling, the mixture was acidified with dilute hydrochloric acid and filtered. The filtrate was extracted with ether and the precipitate was also washed with ether. The combined ether solution was washed with dilute hydrochloric acid, with aqueous sodium carbonate solution, and finally with water. The ether solution being dried over sodium sulfate and evaporated, the residual product was purified from aqueous methanol and then ethyl acetate to 4',5,7-trihydroxy-8-methoxyisoflavone (0.1 g.) in colorles needles, m. p. 241—2°, having a green ferric reaction in alcohol.

Anal. Found: C, 63.97; H, 4.11. Calcd. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03%.

Acetylation of this isoflavone by the acetic anhydride-pyridine method gave rise to its acetate which was purified from aqueous methanol in colorless needles, m. p. 167°, having a negative ferric reaction in alcohol.

Anal. Found: C, 62.36; H, 4.29. Calcd. for $C_{22}H_{18}O_9$: C, 61.97; H, 4.26%.

Method (b).—A mixture of 4',7-dihydroxy-5,8-dimethoxyisoflavone (0.1 g.) and aluminium chloride (0.2 g.) in nitrobenzene (10 ml.) was heated on a steam-bath for an hour. After cooling, ice-water was added and the nitrobenzene was removed by the steam distillation. The precipitate was filtered, and the unchanged substance (50 mg.) was recovered. By acidification of the filtrate with dilute hydrochloric acid, a crystalline precipitate, was separated. Purification from aqueous methanol gave 4',5,7-trihydroxy-8-methoxyisoflavone (30 mg.), colorless needles m. p. 238—9°. The mixed melting point with the product prepared by method (a) showed no depression.

Anal. Found: C, 63.56; H, 4.54. Calcd. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03%.

We are grateful to the Institute of Agricultural Chemistry, Faculty of Agriculture, Kyoto University for microanalyses.

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Toyama University, Toyama

6) T. A. Geissman and T. G. Halsall, *J. Am. Chem. Soc.*, **73**, 1280 (1951).

Crystallization of Quartz at High Temperatures

By Taneki TOKUDA

(Received March 8, 1957)

It has generally been accepted that amorphous silica[1] and vitreous silica[2] crystallize into cristobalite when they are heated in the absence of a flux. It has also been reported that a mixture of quartz and cristobalite was obtained[3] when amorphous silica was heated at 1200—1300°C with addition of a mineralizer which did not contain alkali or strontium compounds, although at these temperatures quartz is unstable*. Crystallization of quartz as a single phase has been described by Wittels[4], who obtained it by annealing,at 930°C for 16 hours, metamict silica produced by fast neutron bombardment (2×10^{20}neutrons/cm).

It has become evident that the presence of a very small amount of alkali plays an important role in the crystallization of silica, whereas the previous workers do not seem to have paid attention on this point. Therefore, the author has reinvestigated the crystallization of silica at high temperatures using amorphous silica which is free from alkali. It was found that quartz was obtained as a single phase even at 1388±5°C.

Experimental

"Aerosil", amorphous silica, made by DEGUSSA was used as the starting material. Chemical analysis and physical constants of this material are shown in Table I. No trace of alkali was found by means of a chemical analysis. The specimen was held in a platinum boat, and was heated at a given temperature (±5°C) in an electric tube furnance, and was very rapidly cooled in air. No flux was added. The cooled

TABLE I
CHEMICAL ANALYSIS AND PHYSICAL CONSTANTS OF UNFIRED SILICA SPECIMEN

SiO_2	99.9%
Fe_2O_3	0.004%
CaO	None
MgO	None
Free moisture	1.72%
Ignition loss (=Total H_2O)	4.2%
Specific density	2.2
Bulk density	0.050

TABLE II
FIRED SILICA SPECIMEN

Heating Temperature (°C)	Heating hours (hr.)	Cristobalite (111) Intensity (Counts/sec)	Cristobalite (111) Diffraction angle (2θ) (°)	Cristobalite (111) Spacing (Å)	Quartz (101) Intensity (Counts/sec)	Quartz (101) Diffraction angle (2θ) (°)
ca. 1600	5	ca. 2000	22.06	4.029	0	—
1453	1/2	16	22.03	4.034	0	—
1413	1	26	21.96	4.047	0	—
1399	2	28	21.95	4.050	0	—
1392	2	35	21.92	4.054	0	—
1388	1	0	—	—	29	26.65
1374	1	0	—	—	16	26.65
1373	1	0	—	—	0	—
1362	1	0	—	—	0	—
1352	1	0	—	—	0	—

* According to Flörke[7] and Scairer and Yagi[8], quartz is unstable above ca. 1050°C, while according to Fenner[5] it is unstable above 870°C.

1) R. B. Sosman, "The Properties of Silica", The Chemical Catalog Co., Inc., New York, 1927, p. 171.
2) ibid., p. 97.
3) J. H. Schulman, E. W. Claffy and R. J. Ginter, *Am. Mineral.*, **34**, 68 (1949); D. A. Bailey, ibid. **34**, 601(1949); L. S. Birks and J. H. Schulman, ibid. **35**, 1035 (1950); G. D. Rieck and J. M. Stevels, *J. Soc. Glass Technol.*, **35**, 284 (1951).
4) M. Wittels and F. A. Sherrill, *Phys. Rev.*, **93**, 1117 (1954).
5) C. N. Fenner, *Am. J. Sci.*, **36**, 331 (1913).
6) T. Tokuda, *Mem. Inst. Sci. Ind. Res. Osaka Univ.*, **14** (1957) in press.
7) O. W. Flörke, *Naturwiss.*, **43**, 419 (1956).
8) J. F. Scairer and K. Yagi, *Am. J. Sci.*, Bowen Vol., 500 (1952).

specimens were analysed by the "Norelco" X-ray diffractometer. Filtered Cu—K_α was used with an X-ray tube running at 30 kV, 15 mA. Divergence and scatter slits were 1°, and the receiving slit was 0.006" in width respectively.

Results

The reflection (111) for cristobalite was detected along with the amorphous band with specimens which were heated at 1392, 1399, 1413, or 1453°C for one hour (Fig. 1). The reflection (101) for quartz was detected along with the amorphous band with those which were heated at 1388 or 1374°C for one hour (Fig. 2). These are the highest temperatures at which quartz has been crystallized, reported in the literature. The diffraction angle for (101) of quartz of these products is, as is shown in Table II, equal to that of a large transparent Brazilian quartz within an experimental error (0.01°). On the other hand, it was found that, for crisiobalite, as the temperature of heating is lower, the products show larger spacing. Thus a specimen which was heated at 1392°C±5 for one hour showed a spacing for cristobalite 0.6% larger compared to that of the standard specimen which was obtained by heating amorphous silica at ca. 1600°C for five hours.

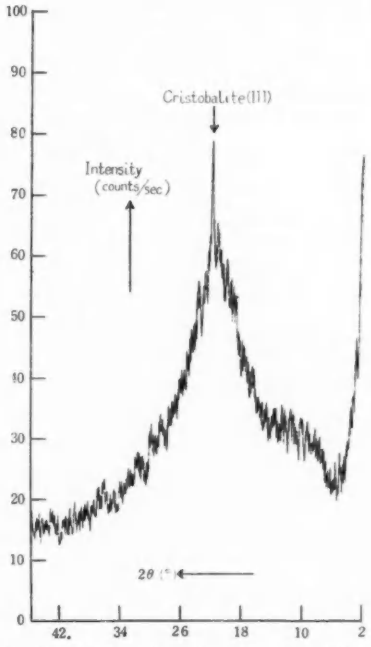


Fig. 1. X-ray diffraction powder pattern of amorphous silica heated at 1413±5°C for one hour in air, showing the appearance of cristobalite.

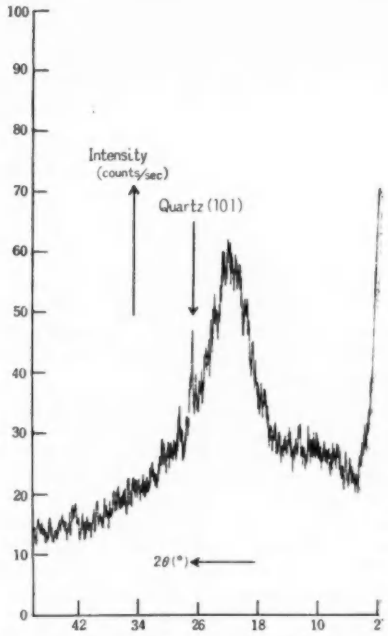


Fig. 2. X-ray diffraction powder pattern of amorphous silica heated at 1388±5°C for one hour in air, showing the appearance of quartz.

Osaka University, Osaka

On 2-Arylaminotropones. II[1]*. Bromination of 2-Anilino- and 2-(p-Toluidino)tropones*[2]

By Syun ISEDA

(Received March 22, 1957)

The present paper describes the bromination of 2-anilino- and 2-(*p*-toluidino)-tropone, reported in Part I of this series[1], together with discussions on related matter.

Bromination in glacial acetic acid, in the presence of anhydrous sodium acetate, of 2-anilinotropone affords, irrespective of the amount of bromine added, a tribromo derivative (I) of m.p. 262.8°C*, and the same of 2-(*p*-toluidino)-tropone affords a dibromo derivative (II), m.p. 180–181°C or a tribromo derivative (III), m.p. 190°C, with a given amount of bromine. In the absence of sodium acetate, I is obtained from 2-anilinotropone and III from 2-(*p*-toluidino)tropone.

In order to establish the structure of these compounds, their hydrolysis was attempted by heating with 4N ethanolic potassium hydroxide. I afforded *p*-bromoaniline(N-acetyl derivative, m.p. 163.5°C) as a basic component and 3,5-dibromotropolone, m.p. 154°C(*p*-toluidine salt, m.p. 122.5°C)[3] as an acidic component. II formed *p*-toluidine (N-acetyl derivative, m.p. 153°C) and 3,5-dibromotropolone. III remained inert on refluxing with 2N ethanolic potassium hydroxide for 30 minutes, almost all the starting material being recovered. Its ultraviolet absorption spectrum (Fig. 1)[4] indicated the presence of a tropone ring and that aromatization had not taken place.

When III was refluxed with 6N ethanolic potassium hydroxide for 6—8 hours or with sodium methoxide in methanol for 3 hours, it was separated into a basic component IV (N-acetyl derivative, m.p. 118–119°C) and an acidic component.

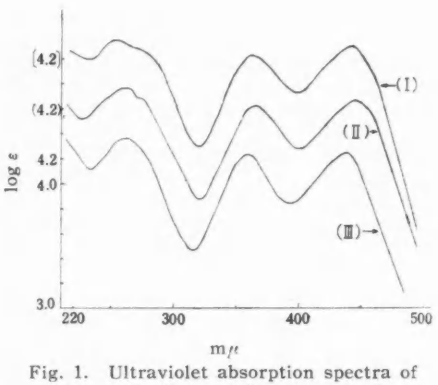


Fig. 1. Ultraviolet absorption spectra of (III), (II) (log ε+0.4), and (I) (log ε +0.8) (in methanol).

Vacuum sublimation of this acidic component yielded a small amount of 3,5-dibromotropolone, indicating that no additional bromine substitution of the tropone ring had taken place and that the substitution occurred in the benzenoid portion. It is therefore assumed that IV is a bromo-*p*-toluidine and that the bromine had entered into the position *meta* to the methyl group in *p*-toluidine, in accordance with the results of bromination of *p*-acetotoluidide[5] and 3-nitrosalicylic acid *p*-toluidide[6]. Comparison with an authentic specimen proved IV to be 4-amino-3-bromotoluene.

It can be concluded from the foregoing results that I is 2-(4′-bromoanilino)-5,7-dibromotropone, and II and III are respectively 2-(*p*-toluidino)- and 2-(2′-bromo-*p*-toluidino)-5,7-dibromotropone. The ultraviolet absorption spectra of I, II, and III are shown in Fig. 1.

Their infrared spectra[7] all indicated the absorption of 1,4-disubstituted benzene

1) Part I: S. Iseda, *Bull. Chem. Soc. Japan*, **28**, 617 (1955).

2) Presented at the Kyushu Local Meeting of the Chemical Society of Japan, Kumamoto, December 4, 1954.

* All melting points corrected.

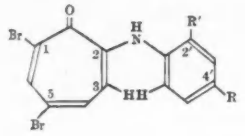
3) T. Nozoe, Y. Kitahara, K. Yamane and A. Yoshikoshi, *Proc. Japan Acad.*, **27**, 18 (1951).

4) Ultraviolet absorption spectra were determined by a Beckman spectrophotometer by members of the Chemistry Department, Faculty of Science, Tohoku University, Sendai.

5) "Organic Syntheses", Coll. Vol. I (Japanese translation), p. 113 (1935).

6) G. V. Jadhav and P. G. Nerlekar, *J. Indian Chem. Soc.*, **29**, 765 (1952); *J. Univ. Bombay*, **20**, part 3, 47 (1951).

7) Infrared spectra were determined by Messrs. H. Kozuma and K. Kawasaki, Technical Department, Shin Nippon Chisso Hiryo K. K., Minamata.



	R	R′
(I)	Br	H
(II)	CH_3	H
(III)	CH_3	Br

TABLE I

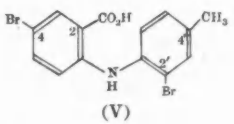
WAVE LENGTH OF ABSORPTIONS OF 1,4-DISUBSTITUTED BENZENE AT 8–10 μ region. (Unit in microns).

I	8.28	9.15	9.59
II	8.29	9.16	9.62
III	8.25	9.15	9.62

(Table I), while that of III indicated the additional absorption at 8.74 μ characteristic of 1,2,4-trisubstituted benzene[8].

The substitution of bromine in the tropone ring of 2-aminotropone itself occurs in the order of C_7, C_5 and C_3[9]. In the present series of experiments, no significant difference was observed between C_7 and C_5 but the third bromine atom entered the benzene ring, instead of the tropone ring. This indicates that the C_3 position of the tropone ring in 2-arylaminotropone suffers the steric hindrance of the arylamino group while such a hindrance does not exist in the 2-aminotropone [see constitution of I].

Application of sodium methoxide to III yielded a small amount of 4-amino-3-bromotoluene and 3,5-dibromotropolone, and most part (73% of the starting material) of the product turned to yellow prisms (V), m.p. 247°C, $C_{14}H_{11}O_2NBr_2$. V is negative to the ferric chloride test and forms a sodium salt of pale yellow needles, sparingly soluble in water. Application of diazomethane to V easily affords its methyl derivative VI, m.p. 93–94°C, $C_{15}H_{13}O_2NBr_2$. The infrared spectra of V and VI (Fig. 2) indicate the presence of a carboxyl(5.93μ) in V and an ester(5.85μ) in VI, and their ultraviolet spectra (Fig. 3) are notable in that the absorption in the wave length region longer than 420 mμ has disappeared. The probable constitution of



(V)

V, 2-carboxy-2′, 4-dibromo-4′methyldiphenylamine, is now under study by synthesis.

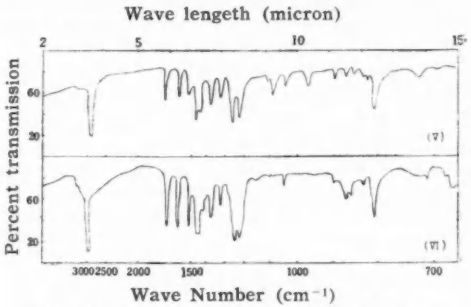


Fig. 2. Infrared absorption spectra of (V) and (VI) (in Nujol).

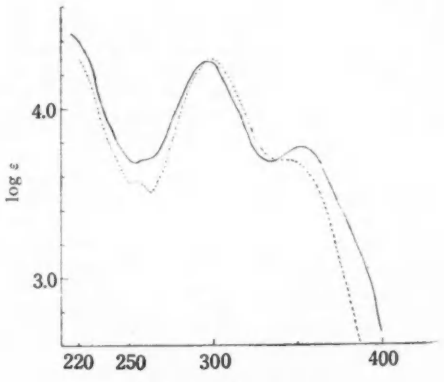


Fig. 3A. Ultraviolet absorption spectra of (V) in neutral (——), and in alkaline medium (·····).

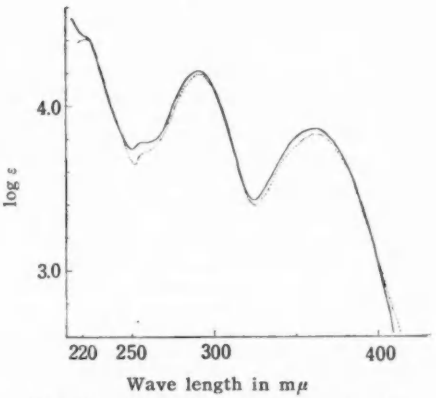
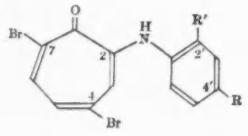


Fig. 3B. Ultraviolet absorption spectra of (VI) in neutral (——), and in alkaline medium (·····).
(Note. Neutral: in methanol. Alkaline: in 0.1 N NaOH in methanol).

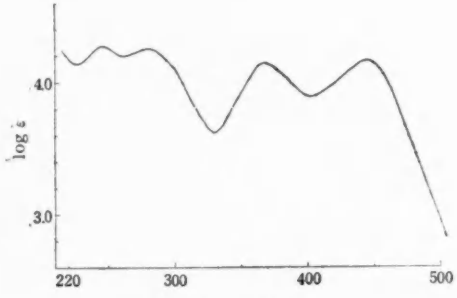
8) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules", p. 70 (1954).

9) T. Nozoe, S. Seto, H. Takeda, S. Morosawa and K. Matsumoto, *Proc. Japan Acad.*, **27**, 556 (1951); **28**, 192 (1952); *Sci. Repts Tohoku Univ.*, Ser., I, **36**, 126 (1952).

Bromination of 2-anilino- and 2-(*p*-toluidino)-4,7-dibromotropone[2] in glacial acetic acid, with or without the addition of sodium acetate, resulted in an introduction of one atom of bromine in both cases and respectively afforded orange yellow prismatic needles, (VII), m.p. 294.5°C, $C_{13}H_8ONBr_3$, and VIII, m.p. 264°C, $C_{14}H_{10}ONBr_3$. Since the newly introduced bromine atom is not in the form of a salt, the following structure in which the substitution has occurred in the benzene ring, can be assumed. This structure is supported by the fact that its ultraviolet spectra are similar to those of I and III. (See Fig. 4.)



	R	R'
(VII)	Br	H
(VIII)	CH_3	Br



Wave length in mμ

Fig. 4. Ultraviolet absorption spectra of (VII) (in methanol).

Experimental

2-(4'-Bromoanilino)-5,7-dibromotropone (I)—Bromination of 2-anilinotropone gave the same results whether sodium acetate was added or not, but when it was added, some complications arose in making the product completely free of ash. A solution of 600 mg. (0.003 mole) of 2-anilinotropone dissolved in 5.0 cc. of glacial acetic acid was treated with 1020 mg. (0.0063 mole) of bromine[10] under stirring and the yellow precipitate that began to form a few minutes later was collected by filtration after 3 hours. Yield, 510 mg.

The mother liquor failed to yield any more crystals on further standing for 2 days, but precipitated crystals immediately on the addition of further 510 mg. of bromine. Total yield, 1260 mg. (almost 100% of the calculated amount). The two lots of precipitate were each dried over sodium hydroxide in vacuo, weighed and recrystallized successively from ethyl acetate and benzene. Both formed the same yellow prisms, m.p. 262–262.8°C (sealed tube), and their admixture indicated them to be an identical substance.

Anal. Found: C, 36.15; H, 1.94%. Calcd. for $C_{13}H_8ONBr_3$: C, 35.96; H, 1.86%.

The same product was obtained whether 3.5 molar equivalents of bromine were added at once or 4–5 molar equivalents. In the case of the latter, some red prisms were found to be mixed in the yellow crystals but these turned yellow during suctional filtration. The crystals of m.p. 262–262.8°C remained unchanged on heating in 5% aqueous ammonia or on shaking of its benzene solution with 2% sodium hydroxide. Its infrared spectrum also failed to exhibit the absorption of $>C=N^+<$ at around 5.9 μ.

Bromination of 2-(*p*-Toluidino)tropone.—a) *With the addition of sodium acetate*: To a solution of 210 mg. (0.001 mole) of 2-(*p*-toluidino)-tropone dissolved in 4.0 cc. of glacial acetic acid and containing 0.5 g. of anhydrous sodium acetate, 180 mg. (0.0011 mole) of bromine was added while stirring under chilling in ice water, by which crystals began to precipitate out after a few minutes. After allowing the mixture to stand overnight with occasional agitation, the precipitate was collected by filtration, dried over sodium hydroxide in vacuo, and 210 mg. of the product was dissolved in 50 cc. of benzene. The benzene solution was washed consecutively with water, diluted hydrochloric acid, dilute sodium hydroxide solution, and water, dried over anhydrous sodium sulfate, and the solvent was distilled off. The residue was recrystallized three times from ethyl acetate and afforded 2-(*p*-toluidino)-5,7-dibromotropone (II) as lemon-yellow needles, m.p. 180–181°C.

Anal. Found: C, 45.57; H, 3.25; N, 3.87%. Calcd. for $C_{14}H_{11}ONBr_2$: C, 45.54; H, 3.01; N, 3.79%.

Crystals did not appear on standing of the mother liquor of II for one week and addition of 320 mg. (0.002 mole) of bromine gradually precipitated crystals. The mixture was allowed to stand at 30°C for 40 hours and the precipitate was purified as for II, affording 180 mg. of 2-(2'-brome-*p*-toluidino)-5,7-dibromotropone (III). Recrystallization from ethyl acetate gave lemon-yellow needles, m.p. 190°C, which melted at 150–153°C on admixture with II.

Anal. Found: C, 37.94; H, 2.44; N, 3.23%. Calcd. for $C_{14}H_{10}ONBr_3$: C, 37.52; H, 2.25; N, 3.13%.

Addition of water to the glacial acetic acid mother liquor of III only formed a minute amount of reddish brown resinous matter.

b) *Without the addition of sodium acetate*: To a stirred solution of 210 mg. of 2-(*p*-toluidino)-tropone dissolved in 4.0 cc. of gracial acetic acid, 500 mg. (0.0031 mole) of bromine was added under cooling and crystals began to precipitate out gradually after about one hour. After this

10) All the bromine used in this experiment was a glacial acetic acid solution containing 100 mg. of bromine in 1.0 cc. of the solution.

11) The microanalyses were carried out at the Institute of Polytechnics, Osaka City University, and at the Pharmaceutical Department, Kumamoto University.

mixture being set aside for 5 hours with occasional agitation, the yellow crystals mixed with yellowish brown to reddish brown crystals were collected by suctional filtration by which all the crystals turned yellow. Yield, 400 mg. (88 %). After drying, the crystals were recrystallized twice from ethyl acetate to yellow needles, m.p. 190°C, identical with III.

When 180 mg. (0.0011 mole) of bromine was added under the same conditions, the crystals failed to precipitate out even after a few days. When a further amount of bromine (220 mg.) was added, or 400 mg. (0.0025 mole) of bromine was added at first, 330 mg. of III, m.p. 190°C, was obtained.

Decomposition of 2-(4'-Bromoanilino)-5,7-dibromotropone (I).—A mixture of I (500 mg.) and 40 cc. of 4 N ethanolic solution of sodium hydroxide was refluxed for 4 hours, the solvent was distilled off under a slight suction, and the distillate was collected in a receiver containing a small amount of hydrochloric acid. The residue in the distillation flask was mixed with water, and the mixture (A) was extracted with ether. The combined distillate and the ether extract was adjusted to pH 3.0 with hydrochloric acid and evaporated to dryness. A few drops of acetic anhydride was added to the residue, allowed to stand over night, mixed with water and again evaporated to dryness. The residue was recrystallized from aqueous ethanol and 25 mg. of *p*-bromoacetanilide was obtained as colorless needles, m.p. and mixed m.p. 161°C.

The alkaline aqueous solution (A) left after shaking with ether was acidified with hydrochloric acid, extracted with ether, and the ether was evaporated. Vacuum sublimation of the residue resulted in the sublimation of ca. 1/4 of the substance and 55 mg. of pale yellow crystals, giving positive ferric chloride reaction, was obtained. Recrystallization from dilute ethanol with charcoal afforded crystals of m.p. 152°C, which formed a *p*-toluidine salt, m.p. 122°C, by the usual method. No depression of the melting point occurred on admixture with 3,5-dibromotropolone.

Decomposition of 2-(*p*-Toluidino)-5,7-dibromotropone (II).—The sample (400 mg.) was refluxed for 6 hours as with I to effect hydrolysis and 20 mg. of *p*-acetotoluidide, m.p. 151°C and 30 mg. of 3,5-dibromotropolone were obtained.

Reaction of 2-(2'-Bromo-*p*-toluidino)-5,7-dibromotropone (III) and 2N Sodium Hydroxide.—A mixture of 300 mg. of (III) and 30 cc. of 2N ethanolic sodium hydroxide solution was refluxed for 30 minutes; cooling of the clear reaction mixture precipitated crystals. Recrystallization from ethyl acetate afforded crystals m.p. 186–188°C, undepressed on admixture with III. Rate of recovery, ca. 160 mg.

Reaction of (III) and Sodium Methoxide.—A mixture of 300 mg. of III and sodium methoxide solution (2g. of metallic sodium dissolved in 50cc. of absolute methanol) was refluxed for 3 hours. the most part of the solvent was distilled off under a reduced pressure, and the residue was treated as for I, affording 10 mg. of a basic component. Its acetylation and recrystallization of the product from dilute ethanol gave the N-acetate as fine needles, m.p. 119–120°C, undepressed on admixture with the synthesized 4-acetamino-3-bromotoluene, m.p. 119–120°C. This substance melted at 90–93°C when admixed with 4-acetamino-2-bromotoluene, m.p. 112–113°C[12]. 4-Acetamino-2-bromotoluene was obtained through the diazotation of 2-amino-4-nitrotoluene to 2-bromo-4-nitrotoluene, followed by reduction with tin and hydrochloric acid, and application of acetic anhydride to the amine so obtained, at room temperature.

The acidic component obtained from this hydrolysis was warmed with 4 cc. of ethyl acetate and the solution was allowed to stand for a few days, depositing 220 mg. of crystals. The ethyl acetate mother liquor was evaporated to dryness and the residue (30 mg.) was submitted to vacuum sublimation, from which ca. 8 mg. of 3,5-dibromotropolone, m.p. 152°C, was obtained. The sublimation residue was an acidic, amorphous brown mass. Crude crystals (220 mg.) were recrystallized several times from 80 % ethanol, using charcoal a few times, to lustrous yellow needles, m.p. 247°C (V).

Anal. Found: C, 44.00; H, 2.91 %. Calcd. for $C_{14}H_{11}O_2NBr_2$: C, 43.66; H, 2.88 %.

This substance gives negative ferric chloride reaction in ethanol and forms a sodium salt of almost colorless needles, somewhat sparingly soluble in water, with sodium bicarbonate solution. This sodium salt melts once at 95°C, solidified again, and does not melt up to 275°C. The sodium salt regenerates the original substance, m.p. 246–247°C, by the action of hydrochloric acid.

An excess of diazomethane in ether was added to 150 mg. of the substance of m.p. 247°C suspended in 30 cc. of ether, by which the crystals gradually disappeared. After 3 hours, the remaining diazomethane was decomposed with acetic acid and recrystallization of the product from 85 % ethanol afforded the methyl ester (VI) as pale yellow needles, m.p. 93–94°C. Maximum absorption in Nujol: 5.85 μ (ester carbonyl). The analytical values corresponded to those of the monomethyl derivative.

Anal. Found: C, 45.35; H, 3.39 %. Calcd. for $C_{15}H_{13}O_2NBr_2$: C, 45.15; H, 3.28 %.

2-(4'-Bromoanilino)-4,7-dibromotropone (VII).—A mixture of 0.7 g. of 2-anilino-4,7-dibromotropone and 60 cc. of glacial acetic acid, containing 2 g. of anhydrous sodium acetate, was heated, cooled, and 500 mg. of bromine was added to the crystalline brei. After a little time, yellow crystals began to precipitate out. The mixture was allowed to stand over night and the crystals were recrystallized from benzene or ethyl acetate, affording 0.50 g. of (VII) as reddish yellow prisms, m.p. 294–295°C.

Anal. Found: C, 35.90; H, 1.90 %. Calcd. for $C_{13}H_7ONBr_3$: C, 35.96; H, 1.86 %.

12) J. J. Blanksma, *Chem. Weekbl.*, **6**, 899 (1909); *Chem. Zentr.*, **1910**, I, 260.

2-(2'-Bromo-*p*-toluidino)-4,7-dibromotropone (VIII).—This was prepared from 1.2 g. of 2-(*p*-toluidino)-4,7-dibromotropone by the method used for that of the aniline analog and recrystallization from benzene afforded 1.2 g. of (VIII) as yellow prisms with a slight reddish tint, m.p. 264°C.

Anal. Found: C, 37.47; H, 2.30%. Calcd. for $C_{14}H_{10}ONBr_3$: C, 37.52; H, 2.25%.

Concentration of the benzene mother liquor yielded 0.14 g. of yellow fine prisms, m.p. 213–215°C.

A part of the experiment reported herein was carried out at the Chemistry Department Faculty of Science, Tohoku University, Sendai, and the writer takes this opportunity to express his sincere gratitude to Professor Tetsuo Nozoe for his kind guidance and encouragement throughout the course of this work. The writer is also indebted to those who supplied analytical data.

The present series of studies constitute a part of the general research on azulenoid compounds (Representative: Professor T. Nozoe). Part of the expenses for the present work was defrayed by a Grant in Aid for Scientific Research provided by the Ministry of Education, which is gratefully acknowledged.

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A Simplified Method for the Preparation of N-Acetylphenylalanines

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Among various synthetic preparations of phenylalanine and its nuclear-substituted derivatives the Erlenmeyer's azlactone method[1,2] seems to be the most accepted one. During the course of the study on the synthesis of aromatic amino acids by this method, a simple and practical procedure has been found in a step of the reductive cleavage of the azlactone ring to yield N-acetylphenylalanines. The application of this method to several azlactones is described in this communication.

It is well-known that benzoyl- or acetylglycine, when heated with aromatic aldehyde in acetic anhydride in the presence of fused sodium acetate as condensing catalyst, yields 2-phenyl- or 2-methyl-4-benzal-5-oxazolone respectively. The route to N-acetylphenylalanines from the azlactones that has ever been utilized is as follows: neutral, alkaline or acidic hydrolysis of azlactones and subsequent hydrogenation of the resulting acetylaminocinnamic acids to acetylphenylalanines. The reducing agents which have been used for this conversion are: 1. Sodium[3,4] or sodium amalgam and water or ethanol. 2. Catalytic hydrogenation (Pt, Pd[5] or Raney nickel[6]).

Since N-acetylphenylalanines were found to be susceptible to optical resolution[7,8] it seemed worthy to study to find a good and simple preparative method for these compounds.

Oxazolones were found to be easily reduced in a dilute aqueous solution of an equivalent amount of sodium hydroxide under pressure using Raney nickel catalyst. The reduction proceeded very smoothly at lower temperature and at the same time the cleavage of the azlactone ring occurred to form sodium salts of N-acetylphenylalanines during the course of hydrogenation. The reduction of the C=C double bond may be facilitated by the presence of alkali which also naturally acts as hydrolyzing agent. By acidification of the reduction mixture with concentrated hydrochloric acid N-acetylphenylalanines were readily obtained.

1) E. Erlenmeyer, *Ann.*, **275**, 1 (1893).
2) H. E. Carter, "Organic Reactions", Vol. 3, 198, John Wiley & Sons Inc., New York, N. Y., 1946.
3) G. Barger and A. J. Ewins, *Biochem. J.*, **11**, 58 (1917).
4) A. Ellinger and Z. Matsuoka, *Z. Physiol. Chem.*, **91**, 45 (1914).
5) R. M. Herbst and D. Shemin, "Organic Syntheses", Coll. Vol. 2, 491, John Wiley & Sons Inc., New York, N. Y., 1943.
6) J. Elks, D. F. Eliott and B. A. Hems, *J. Chem. Soc.*, **1944**, 629.
7) N. F. Albertson, *J. Am. Chem. Soc.*, **73**, 452 (1951).
8) D. G. Doherty and E. A. Pope, *J. Biol. Chem.*, **189**, 447 (1951).

TABLE I

N-ACETYL-β-(SUBSTITUTED PHENYL)-ALANINES

Substituted Group	Yield[a], %	m.p.[b], °C	Formula	C % Calcd.	C % Found	H % Calcd.	H % Found	N % Calcd.	N % Found
none	98	144	$C_{11}H_{13}O_3N$	63.77	63.56	6.28	5.91	6.76	6.62
p-methyl	99	164	$C_{12}H_{15}O_3N$	65.16	65.65	6.79	6.81	6.33	6.04
p-methoxy	96	150	$C_{12}H_{15}O_4N$	60.76	60.71	6.33	6.29	5.91	5.69
3,4-methylenedioxy	92	164—6	$C_{12}H_{13}O_5N$	57.37	57.31	5.18	5.17	5.58	5.51

a) Yields are based upon weight of crude product since in general no purification was required before hydrolysis or resolution.

b) Melting point of the analytical sample.

Four azlactones prepared from acetylglycine, benzaldehyde, tolualdehyde, anisaldehyde and piperonal by usual method were all converted in to the corresponding N-acetylphenylalanines in almost quantitative yield by the procedure described above. The results are summarized in Table I.

It was also found that 2-phenyl-5-oxazolone was very resistant to this hydrogenation unlike 2-methyl-5-oxazolone.

From the standpoint of preparation, the above procedure is characterized by excellent yield and readiness of operation.

Experimental

2-Methyl-4-benzal-5-oxazolone.—This azlactone was prepared according to Herbst's procedure[5].

2-Methyl-4-(*p*-methylbenzal)-5-oxazolone.—By analogous procedure given for 2-methyl-4-benzal-5-oxazolone this compound was prepared. The experimental conditions are as follows: a mixture of 165 g. of acetylglycine, 97.5 g. of fused sodium acetate, 250g. of freshly distilled *p*-methylbenzaldehyde, and 380 g. of 90 % acetic anhydride was warmed until the solution was complete. The resulting solution was then heated for one hour under reflux, cooled and placed in a refrigerator overnight. The brown solid mass obtained was digested with cold water. The yellow crystals melting at 133—135°C weighed 188 g. An analytical sample was obtained by recrystallization from chloroform and petroleum ether.

Anal. Found: C, 71.54; H, 5.42; N, 6.83%. Calcd. for $C_{12}H_{11}O_2N$: C, 71.64; H, 5.47; N, 6.96%.

2-Methyl-4-(*p*-methoxybenzal)-5-oxazolone.—A mixture of 54.4 g. of anisaldehyde, 38.6 g. of acetylglycine and 19.7 g. of anhydrous sodium acetate in 82 ml. of acetic anhydride was treated in the same manner. Thirty grams of yellow crystals with melting point of 114°C was obtained. The azlactone was recrystallized from chloroform-petroleum ether.

Anal. Found: C, 66.65; H, 5.26; N, 6.34%. Calcd. for $C_{12}H_{11}O_3N$: C, 66.36; H, 5.07; N, 6.45%.

2-Methyl-4-(3', 4'-methylenedioxybenzal)-5-oxazolone.— With 37.5 g. of piperonal, 23.4 g. of acetylglycine, 12.3 g. of sodium acetate and 52 ml. of acetic anhydride, 46.2 g. of the azlactone was obtained as yellowish crystals melting at 176—180°C. An analytical sample was obtained by recrystallization from acetic acid and petroleum ether.

Anal. Found: N, 5.77%. Calcd. for $C_{12}H_9O_4N$: N, 6.06%.

General Procedure of Preparation of N-Acetylphenylalanines.—Four azlactones prepared as described above were all converted into the corresponding N-acetylphenylalanines in the following manner: one mole of the crude benzaloxazolone, an about five per cent. aqueous solution of an equivalent amount of sodium hydroxide and Raney nickel catalyst (as alloy, one tenth or one twentieth of the oxazolone used, by weight) were placed in a stainless steel autoclave. An initial hydrogen pressure of 40—70 atm. was applied and the apparatus was shaken at room temperature or, if necessary, at a higher temperature of 30—50°C. Hydrogen was absorbed rapidly in most cases. When, after 0.2—2 hours, one mole of hydrogen had been absorbed, the reaction was stopped and the catalyst was removed by filtration. The pH of the clear orange-yellow filtrate was adjusted to 2—3 with concentrated hydrochloric acid. After being kept overnight in an ice-box, the crystals were collected and washed with water. The crude products thus obtained could be recrystallized from hot water. Table I shows the results of yield, melting point and elementary analysis of four N-acetylphenylalanines.

Summary

A simple and practical procedure is described of preparing N-acetylphenylalanines by hydrogenation of the corresponding unsaturated azlactones in an aqueous sodium hydroxide solution with Raney nickel under pressure. By this method N-acetyl derivatives of phenylalanine, *p*-methylphenylalanine, *p*-methoxyphenylalanine and 3,4-methylenedioxyphenylalanine were synthesized in good yield.

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Synthesis of β- and γ-Thujaplicins

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(Received March 22, 1957)

Synthesis of α-, β- and γ-thujaplicins were reported by Nozoe[1] in 1950 and by Cook[2] in 1951. These methods were started with the preparation of the three isomers of Isopropylcycloheptanone by cyclization or ring enlargement, followed by oxidation to the dione compounds. Dehydrobromination after bromination afforded isomeric thujaplicins.

The present writers synthesized β-thujaplicins by the following two methods (A) and (B), which are herein described.

In the *A* method, perillaldehyde oxime (I) is used as the starting material and this is converted into tetrahydroperillamine (II) and via 4-isopropylcycloheptanone (IV) to the objective VI. The other *B* method starts with 4-isopropylcyclohexanone (VII) which is submitted to ring enlargement with nitromethane and via 4-isopropylcloheptanone to VI. The route of synthesis in this *A* method is shown in Fig. 1.

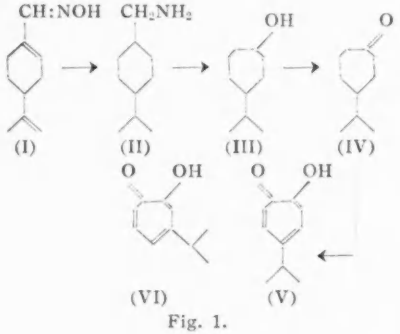

Fig. 1.

In this preparative method, perillartin, the oxime of perillaldehyde, was hydrogenated in ethanolic solution with nickel catalyst at 100°C and 100 atom., for about one hour and tetrahydroperillamine II was obtained in 63% yield. Ring enlargement of II was effected by the method of Ruzicka[3] and of Smith and Baer[4].

We prepared 4-isopropylcycloheptanol (III) in 31% yield by application of nitrous acid to the acetic acid solution of II at 40—50°C and its oxidation by the usual method gave 4-isopropylcycloheptanone (IV) in 50% yield.

Treatment of 4-isopropylcycloheptanone IV by the method of the afore-mentioned Nozoe and Cook, gave a mixture of β- and γ-thujaplicins(V), but the β-isomer was not isolated in pure state and was identified by paper chromatography[5].

The yield by this method of synthesis is 0.4% from the starting material, perillaldoxime, and it cannot be called an advantageous process since in the ring enlargement of perillamine, a fair amount of by-products form besides the objective 4-isopropylcycloheptanol (III), such as tetrahydroperillyl alcohol.

The route of synthesis in the *B* method is shown in Fig. 2.

In this method, the ring enlargement of isopropylcyclohexanone was effected by the nitromethane method of Dauben et al.[6]. The synthesis of thujaplicins from 4-isopropylcyclohexanone VII has not been made by this method as yet. The yield of

1) T. Nozoe, S. Seto and K. Kikuchi, *Proc. Japan Acad.*, **26**, 43 (1950); T. Nozoe, Y. Kitahara and S. Ito, ibid., **26**, 47, (1950); T. Nozoe, S. Seto, K. Kikuchi and H. Takeda, ibid., **27**, 146 (1951).

2) J. W. Cook, R. A. Raphael and A. I. Scott, *J. Chem. Soc.*, **1951** 695.

3) L. Ruzicka and W. Burgger, *Helv. Chim. Acta*, **9**, 399 (1926).

4) P. A. S. Smith and D. R. Baer, *J. Am. Chem. Soc.*, **74**, 6135 (1952).

5) E. Zavarin and A. B. Anderson, *J. Org. Chem.*, **21**, 332 (1956).

6) H. J. Dauben Jr., H. J. Ringold, R. H. Wade and A. G. Anderson, *J. Am. Chem. Soc.*, **73** 2359 (1951).

TABLE I
R_f VALUE OF THUJAPLICINS

Mobile Phase	Stationary Phase (% H_3PO_4)	β-Thujaplicin from Taiwan Hinoki)	γ-Thujaplicin (synthetic)	β- and γ-Thujaplicin mixture	
Ligroine-toluene (3 : 1)	17	0.45	0.32	0.44	0.32

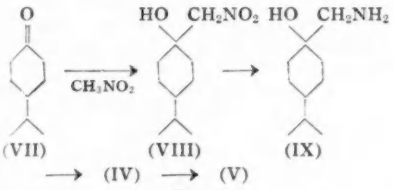

Fig. 2.

IV from VII is about 23.7% and further examination seems required as a synthetic method for thujaplicins. Derivation of IV to thujaplicins followed the same route as in A method. The starting material, 4-isopropylcyclohexanone VII was obtained according to the method described by Frank and Berry[7].

Experimental

Tetrahydroperillamine (II).—A mixture of 33 g. of perillartin (I) in 66 cc. of ethanol was submitted to hydrogenation with Raney nickel as a catalyst, at 100°C and 100 atm.. The catalyst was filtered off, ethanol was distilled off from the filtrate, and the residue was distilled under a reduced pressure, affording 21 g. of II, b. $p._{15}$ 100—115°C n_D^{21} 1.4772

4-Isopropylcycloheptanol (III).—A solution of 21 g. of II, 8.5 g. of glacial acetic acid, and 70 cc. of water was warmed to 40—50°C, and a solution of sodium nitrite in 20 cc. of water was added dropwise during thirty minutes. After stirring this mixture for thirty minutes at this temperature, the oily layer was separated and submitted to steam distillation. The oily distillate was distilled under a reduced pressure. B. $p._{12}$ 95—110°C, yield of III 6.5 g. (31%) n_D^{22} 1.4735.

4-Isopropylcycloheptanone (IV).—A mixture of 6.5 g. of III, 4.5 g. of potassium dichromate, 11 g. of 50% sulfuric acid and 10 g. of benzene was heated for thirty minutes at 50—60°C to effect oxidation and 3.3 g. (50%) of IV was obtained as an oil of b. $p._{15}$ 105—115°C. n_D^{22} 1.4689.

Semicarbazone: m. p. 153-4°C.

Anal. Found: C, 62.59; H, 9.62; N, 19.89%. Calcd. for $C_{11}H_{21}ON_3$: C, 62.6; H, 9.95; N, 19.9%.

Sodium Salt of Bromo-β- and -γ-thujaplicins.—To heated solution of 12 g. of IV dissolved in 25 cc. of dehydrated ethanol, a solution of 10.5 g. of selenium dioxide dissolved in 60 cc. of dehydrated ethanol was added dropwise during two hours and the mixture was stirred at the same temperature for five hours. After standing this over night, the precipitated selenium was filtered off, the filtrate was steam distilled, and the oily residue was extracted with benzene. The benzene extract was washed with water, dried, and benzene distilled off. The residue was distilled under a reduced pressure and 9.5 g. (79%) of a mixture of the diones, b. $p._{16}$ 130—140°C, was obtained. n_D^{25} 1.4730.

A solution of 15 g. of the foregoing dione mixture dissolved in 0.5 cc. of acetic anhydride and 4.5 cc. of glacial acetic acid was chilled to 0°C to 10°C and a solution of 29 g. of bromine in 3 cc. of gracial acetic acid was added dropwise during four hours. The mixture was stirred at this temperature for 1.5 hours, allowed to stand over night (during which a vigorous evolution of hydrogen bromide was observed), and further stirred for four hours while gradually raising the temperature of the reaction mixture to 60°C. The mixture was then submitted to steam distillation, the residual oil was extracted with ether, and a solid substance was filtered off. To this ether extract, 100 cc. of 10% sodium hydroxide solution was added, the crystals that separated out were collected by filtration, and dried, affording 13 g. (86%) of a mixture of sodium salts of bromo-β- and -γ-thujaplicins.

β- and γ-Thujaplicins.—Catalytic reduction of 13 g. of the sodium salt mixture of bromo-β- and γ-thujaplicins in ethanol, with $Pd(OH)_2$—$CaCO_3$ catalyst, was carried out. After filtration of the catalyst, ethanol was removed by low-pressure distillation and about 10 g. of a solid thus obtained was extracted with toluene. The toluene solution was extracted with phosphoric acid, the acid extract was decomposed with water, and further extracted with toluene. After washing with water and drying, toluene was distilled off and the residue was distilled under a reduced pressure. The oily distillate crystallized and was purified from petroleum ether to 50 mg. of γ-thujaplicin, m. p. 78°C. From this mother liquor, 800 mg. of a mixture of β- and γ-thujaplicin was obtained.

Results of paper chromatography of this mixture was as shown in Table I.

1-Nitromethyl-4-isopropyl-1-cyclohexanol (VIII).—A mixture of 150 g. of 4-isopropylcyclohexanone VII (b. $p._{14}$ 90—95°C, n_D^{21} 1.4570. Semicarbazone, m.p. 186—187°C) and 110 g. of nitromethane was added dropwise during 2.5 hours into sodium alkoxide (prepared from 27 g. of metallic sodium and 750 cc. of dehydrated ethanol) while being stirred at 40—43°C and a mixture was stirred for further nine hours at the same temperature. When cooled, the reaction mixture was cooled in

7) R. L. Frank, R. E. Berry and O. L. Shotwell, ibid., **71**, 3889 (1949).

ice, the reaction product was collected by filtration, and dried. About 400 g. of this sodium salt was placed in a three-necked flask, the flask was chilled in ice, and a thoroughly chilled solution of 142 g. of glacial acetic acid in 1200 cc. of water was added under stirring. After stirring this mixture for further thirty minutes (crystals precipitated out during chilling), this was neutralized and an oily layer was separated. The aqueous solution was extracted with ether, the ether extract was combined with the oily layer, dried, and ether distilled off. The residue was distilled off. The residue was distilled under a reduced pressure and 140 g. (93.9%) of VIII, b. p.$_{15}$ 145—8°C was obtained. m. p. 56—58°C, n_D^{20} 1.4786.

Anal. Found: C, 60.58; H, 9.20; N, 7.04%. Calcd. for $C_{19}H_{19}O_3N$: C, 59.7; H, 9.45; N, 6.97%.

From the mother liquor of the sodium salt, 16 g. of the starting material VII was recovered.

1-Aminomethyl-4-isopropyl-1-cyclohexanol Acetate.—Using a stainless steel autoclave of 300-cc. capacity, 41 g. of VIII in 82 cc. of glacial acetic acid was hydrogenated with Raney nickel catalyst, cooling the autoclave at first, gradually warming to the room temperature, and hydrogenated at 30°±2°C for seven hours (absorption of hydrogen, 10.2 l.). After removal of the catalyst by filtration, the solvent was distilled off under a reduced pressure, and the residual oil was cooled with addition of a small amount of ether by which the oil solidified into crystals. The crystals were collected by filtration, the filtrate was diluted with water, and extracted with ether. The aqueous solution was concentrated to a small volume and the foregoing procedure was repeated, isolating a further crop of crystals, m. p. 119—125°C. Total yield of acetate, 38 g. (90%).

About 3 g. of 4-isopropylcyclohexanone VII was obtained from the ether extract, its semicarbazone, m. p. 185—7°C, did not show any melting point depression on admixture with semicarbazone of VII.

4-Isopropylcycloheptanone (IV).—A solution of 50 g. of 1-aminomethyl-4-isopropyl-1-cyclohexanol acetate dissolved in 32 g. of glacial acetic acid was diluted with 52 cc. of water, chilled to 0°C, and 60 g. of sodium nitrite solution was added dropwise during two hours (vigorous effervescence). After stirring for one hour at the same temperature, the reaction mixture was allowed to stand over night, neutralized with sodium bicarbonate, and an oily layer was separated. The oil was steam distilled and the oily distillate was distilled under a reduced pressure, affording 20 g. of a fraction of b. p.$_{17}$ 90—112°C. This was purified through the semicarbazone and decomposed with oxalic acid, b. p.$_{17}$ 108—110°C. Yield 14 g. (28%). n_D^{25} 1.4625. Semicarbazone, m. p. 144—5°C.

Anal. Found: C, 62.75; H, 9.95; N, 20.50%. Calcd. for $C_{11}H_{21}ON_3$: C, 62.6; H, 9.95; N, 19.9%).

The infrared spectrum of IV exhibited an absorption of C=O at 1694 cm^{-1}.

Summary

β- and γ-Thujaplicins were synthesized through 4-isopropylcycloheptanone from perillaldehyde and 4-isopropylcyclohexanone.

Research Laboratory Takasago Perfumery Co., Ltd. Kamata, Tokyo

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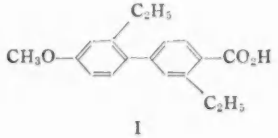
Estrogenic Biphenyls. II. 2',5'-Diethyl-4-methoxybiphenyl-4'-carboxylic Acid

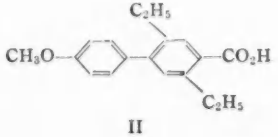
By Michinori ŌKI* and Takeo SATO

(Received April 10, 1957)

In the first paper[1] of the present series it was reported that 2,3'-diethyl-4-methoxybiphenyl-4'-carboxylic acid (I) is estrogenic and the activity can be explained on the basis of the postulation[2] of the suitable distance between the two active hydrogen atoms and the suitable thickness of the molecule for an estrogen.



I



II

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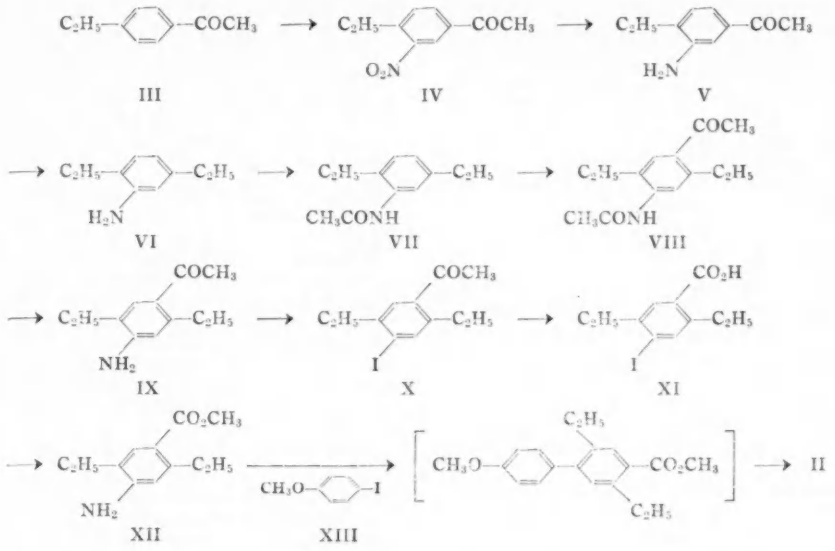
1) M. Ōki and T. Sato, This Bulletin, **30**, 508 (1957).

2) M. Ōki and Y. Urushibara, ibid., **25**, 109 (1952).



It is considered that the suitable thickness of compound I is obtained through the steric effect of the ethyl group at position 2, which causes a deformation of the coplanar structure, and thus increases the molecular thickness of the biphenyl system. As an ethyl group at position 2′ or 6′ should have the same steric effect as the one at position 2, 2′, 5′-diethyl-4-methoxybiphenyl-4′-carboxylic acid (II) has been prepared and tested on estrogenic activity.

Compound II was sythesized in the following way: nitration of 4-ethylacetophenone (III) gave 4-ethyl-3-nitroacetophenone (IV), which was reduced to the corresponding amine (V) with stannous chloride and hydrochloric acid. Modified Wolff-Kischner reduction of compound V yielded 2,5-diethylaniline (VI), of which acetylation product VII showed no depression of the melting point on admixture with the authentic specimen of 2,5-diethylacetanilide[3]. Reduction[3,4] of 2,5-diethylnitrobenzene to VI gave an unsatisfactory result especially on a larger scale, because of the instability of the nitro-compound to heat. Compound VII was treated with acetyl chloride and aluminium chloride and the product, 4-acetamido-2,5-diethylacetophenone (VIII), was hydrolyzed to yield 4-amino-2,5-diethylacetophenone (IX). Compound IX was converted into 2,5-dietyl-4-iodo-acetophenone (X) through a diazo-reaction and the iodo-ketone (X) was oxidized with sodium hypobromite to produce 2,5-diethyl-4-iodo-benzoic acid (XI). Methyl 2,5-diethyl-4-iodo-benzoate (XII) was prepared in the usual way. The ester (XII) and 4-iodoanisole (XIII) were submitted to the Ullmann reaction and the product was hydrolyzed and purified by chromatography through alumina.

TABLE I
ULTRAVIOLET ABSORPTION MAXIMUM

Compound	$\lambda_{max.}$ (mμ)	log $\varepsilon_{max.}$
I	270	4.09
II	268	4.14

The ultraviolet absorption data of compounds I and II are shown in Table I. The absorption spectra of the two compounds were similar and suggested that the deformation in the biphenyl skeletons of these compounds is nearly the same.

Compound II in oil was injected subcutaneously to ovariectomized mice. The minimum active dose to produce full estrus in 100% of the animals was found to be 100 γ and the same as that of compound I[1].

Experimental[5]

4-Ethyl-3-nitroacetophenone (IV).—4-Ethylacetophenone (III) was prepared from ethylbenzene and acetyl chloride according to the method

3) W. J. Gaudion, W. H. Hook and S. G. P. Plant, *J. Chem. Soc.*, **1947** 1631.
4) A. Voswinkel, *Ber.*, **22**, 315 (1889)

5) All melting and boiling points are uncorrected. The authors are indebted to Dr. Y. Osawa of the University of Tokyo for microanalyses.

described by Mowry et. al.[6]. One mole or 148 g. of compound III was dropped into 300 ml. of concentrated sulfuric acid with good stirring, while the temperature was kept below 5° by adding solid carbon dioxide. A mixed acid consisting of 80 ml. of nitric acid (d : 1.42) and 120 ml. of concentrated sulfuric acid was dropped into the sulfuric acid solution of the ketone (III) in forty-five minutes, the temperature being kept below 0° by adding solid carbon dioxide. The mixture was stirred for further ten minutes and poured into 2.5 l. of ice-water. The supernatant layer was decanted off and the lower one was dissolved in ether. The ethereal solution was washed with aqueous sodium hydroxide and then with water and dried over calcium chloride. Fractional distillation gave a pale yellow oil boiling at 135—136°/1 mm. which solidified at 0° and melted at the room temperature. Yield 164 g. or 85%, of the theoretical.

Anal. Found: N, 7.46%. Calcd. for $C_{10}H_{11}O_3N$: N, 7.25%.

3-Amino-4-ethylacetophenone (V). — When a mixture of 193 g. (1 mole) of compound IV, 750 g. (3.3 moles) of stannous chloride and 1.2 l. (6.6 moles) of dilute hydrochloric acid (1 : 1) was stirred, a spontaneous reaction set in and the mixture began to boil. The homogenous solution was allowed to cool and was basified with an excess of 33% aqueous sodium hydroxide. The organic material was extracted with ether and the combined extracts were dried over potassium carbonate. After the solvent was evaporated, the product distilled over at 144-145°/2 mm. as a pale yellow oil. Yield 147 g. or 90% of the theoretical.

Anal. Found: N, 8.76%. Calcd. for $C_{10}H_{13}ON$: N, 8.58%.

2, 5-Diethylaniline (VI).—A solution of 163 g. (1 mole) of the amino-ketone (V), 120 g. (1.6 moles) of 80% hydrazine hydrate and 280 g. of potassium hydroxide in 1 kg. of diethylene glycol was heated under reflux for one hour and the reflux condenser was then replaced by a downward condenser. Distillation was continued until the remainder in the flask attained the temperature of 200°, and it was heated again for thirty minutes. The reaction mixture was cooled and steam-distilled. The oily product was extracted with ether and the extract was dried over potassium hydroxide. Distillation gave a colorless oil boiling at 120—125°/20 mm. Reported boiling points of 2, 5-diethylaniline are 122°/13 mm[3] and 140—142°/20 mm.[4]. Yield 134 g. or 90% of the theoretical. The acetylation product (VII) was recrystallized from aqueous alcohol and melted at 150—151°. Reported melting point of 2, 5-diethylacetanilide (VII) is 154°[3].

4-Acetamido-2, 4-diethylacetophenone (VIII).—To a boiling suspension of 95.5 g. (0.5 mole) of compound VII in 56 g. (0.8 mole) of acetyl chloride and 400 ml. of carbon disulfide, was added 160 g. (1.2 moles) of aluminum chloride with stirring at such a rate that the mixture boiled briskly. The reaction mixture was refluxed with stirring for one hour and then allowed to stand for two hours at the room temperature. The supernatant carbon disulfide layer was decanted off and the lower layer was decomposed by pouring into ice-water in which 200 ml. of concentrated hydrochloric acid had been added. The crystals were collected and recrystallized from aqueous alcohol. Colorless prisms, m.p. 149.5—150°. Yield 100 g. or 90% of the theoretical.

Anal. Found: N, 6.25%. Calcd. for $C_{14}H_{19}O_2N$: N, 6.00%.

4-Amino-2, 5-diethylaetophenone (IX). — A mixture of 111.5 g. (0.5 mole) of the acetyl compound (VIII) and 600 ml. of dilute hydrochloric acid (1 : 1) was heated under reflux for two hours and basified with sodium hydroxide after cooling. The crystals were collected and recrystallized from benzene-petroleum ether. Colorless needles, m. p. 72.5—73°. Yield 71.6 g. or 75% of the theoretical.

Anal. Found: N, 7.38%. Calcd. for $C_{12}H_{17}ON$: N, 7.32%.

2, 5-Diethyl-4-iodoacetophenone (X). — A diazonium salt solution prepared from 95.5 g. (0.5 mole) of compound IX, 150 g. (1.5 moles) of concentrated sulfuric acid, 300 ml. of water and 35 g. (0.5 mole) of sodium nitrite in a minimum quantity of water was gradually added to a warm solution of 166 g. (1 mole) of potassium iodide in 200 ml. of water. The mixture was warmed at 50° for thirty minutes. The heavy oil was extracted with ether and the ethereal extract was washed with water, with aqueous sodium thiosulfate, and again with water, and dried over calcium chloride. Fractional distillation under nitrogen atmosphere gave a pale yellow oil boiling at 136°/2 mm. Yield 91 g. or 60% of the theoretical.

Anal. Found: I, 42.00%. Calcd. For $C_{12}H_{15}OI$: I, 42.29%.

This compound is unstable and quickly discolors on exposure to air. The 2, 4-dinitrophenylhydrazone was recrystallized from alcohol-ethyl acetate. Orange leaflets, m. p. 150—151°.

Anal. Found: N, 11.49%. Calcd. for $C_{17}H_{17}O_4N_4I$: N, 11.62%.

2, 5-Diethyl-4-iodobenzoic Acid (XI). — A solution of 30.2 g. (0.1 mole) of the iodo-ketone (X) in 150 ml. of dioxane was dropped into a sodium hypobromite solution prepared from 56 g. (1.4 moles) of sodium hydroxide, 72 g. (0.45 mole) of bromine and 300 ml. of water in one hour, at the temperature below 10° and with continuous stirring. The excess of sodium hypobromite was decomposed with sodium bisulfite and bromoform was removed by steam-distillation. After cooling, the solution was acidified with hydrochloric acid, and the crystalline substance was recrystallized from aqueous alcohol. Colorless needles, m. p. 134—135°. Yield 25 g. or 82% of the theoretical.

Anal. Found: I, 42.21%. Calcd. for $C_{11}H_{13}O_2I$: I, 41.76%.

Methyl-2, 5-diethyl-4-iodobenzoate (XII).— A solution of 30.1 g. of the acid (XI) in 100 ml. of methanol was saturated with dry hydrogen

6) D. T. Mowry, M. Renoll and W. E. Huber, *J. Am. Chem. Soc.*, **68**, 1105 (1946).

chloride at 0° and the mixture was warmed on a water bath for two hours. The reaction mixture was poured into water and extracted with ether. The extracts were washed with aqueous sodium bicarbonate and dried over potassium carbonate. The ester boiled at 137°/1 mm. Yield 27 g. or 87% of the theoretical.

Anal. Found: I, 40.20%. Calcd. for $C_{12}H_{15}O_2I$: I,39.89%.

4-Methoxy-2',5'-diethylbiphenyl-4'-carboxylic Acid (II).—To a well stirred mixture of 10 g. (0.043 mole) of 4-iodoanisole (XIII), prepared by iodination of anisole[7], and 5 g. (0.016 mole) of compound XII, heated at 240—250°, was added 15 g. (0.235 atom) of copper bronze activated by iodine[8] in portions in twenty minutes. The temperature was then raised to 280° and the mixture was stirred for forty minutes at that temperature. After cooling, the reaction mixture was extracted with acetone and the solvent was evaporated. To the residue was added 50 ml. of 10% sodium hydroxide in 50% aqueous alcohol, and the mixture was heated under reflux for two hours, and then diluted with 500 ml. of water. The insoluble material was filtered off with the aid of active charcoal. The filtrate was acidified with hydrochloric acid and the crystalline substance was collected. The crude acid was dissolved in ether and purified by passing the solution through an alumina column and eluting with the same solvent. Colorless needles, m. p. 161—162°. Yield 0.6 g. or 11% of the theoretical amount based on the used iodoester (XII).

Anal. Found: C, 76.28: H, 7.36%. Calcd. for $C_{19}H_{20}O_3$: C, 76.03: H, 7.09%.

Ultraviolet Absorption Spectra.—The specimens were submitted to measurements in 95% alcohol by the use of Hitachi Photoelectric Spectrophotometer Model EPU-2. 2,3'-Diethyl-4-methoxybiphenyl-4'-carboxylic acid (I) was prepared as described in the previous paper[1].

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7) M. P. Brenans, *Bull. chim. soc. France*, [3] **25**, 819 (1901).
8) E. C. Kleiderer and R. Adams, *J. Am. Chem. Soc.*, **55**, 4225 (1933).

The Bond Refraction Values for Ti-O in Organic Titanates

By Tooru TAKATANI

(Received April 6, 1957)

Many liquid organic titanates are known, but little attention has been paid to the calculation of the molecular refraction values of these compounds.

English and Sommer[1] have recently obtained tetrakis-(trimethylsiloxy)-titane, and have measured its molecular refraction, estimating the bond refraction for Ti-O as 4.02 ml./mol. This seems to be the only example which has appeared in the literature published so far.

For the present paper, the establishment of bond refraction for Ti-O and its application for some organic titanates were undertaken.

Experiment

Ethyl, iso-propyl, and n-butyl titanate were prepared by treating titanium tetrachloride with corresponding alcohol in the presence of ammonia[2].

n-Propyl, sec-butyl, tert-butyl, and tert-pentyl titanate were prepared by the transesterification reaction (1) between ethyl titanate and the corresponding alcohol[3].

$$(C_2H_5O)_4Ti + 4\ ROH = (RO)_4Ti + 4\ C_2H_5OH \quad (1)$$

The preparation of *n-Heptyl titanate* followed a similar method, n-butyl titanate being used in place of ethyl titanate.

Some of the physical properties and titanium contents of the titanates prepared are shown in Table I and II.

Ethyl Titanate.—Ethanol (460 g, 10 mol.) was saturated with ammonia in a flask fitted with a reflux condenser, and all openings were protected against moisture. Titanium tetrachloride (190 g, 1 mol.) was then added drop by drop while

1) W. D. English and L. H. Sommer, *J. Am. Chem. Soc.*, **77**, 170 (1955).

2) J. Nelles, U. S. Patent 2,187,821 (1940), *Chem. Abst.*, **34**, 3764[7] (1940).
3) B. A. Arbuzov and Z. G. Isaeva, *Zhur. Obshchei Khim.* S. S. S. R., **22**, 566 (1952), *Chem. Abst.*, **47**, 2681f (1953).

stirring under cooling by ice during five hours at a temperature below 60°C. After the addition, the mixture was allowed to stand overnight at room temperature. White precipitates of ammonium chloride were removed and the filtrate was heated on a water bath to eliminate the excess of ethanol. The residue was distilled under reduced pressure, to obtain a fraction b.p. 140–145°C/3mmHg (reported, 130–132°C/2mmHg[4]). It was redistilled and used for further experiments.

Anal. Found: Ti, 21.1; C_2H_5O, 79.3. Calcd. for $(C_2H_5O)_4Ti$: Ti, 21.0; C_2H_5O, 79.0%.

Titanium was determined by the Speer's method[5] and the ethoxy group by Bradley's oxidation method[6].

TABLE I

PROPERTIES AND ANALYSES OF THE PREPARED TITANATES

Compd.	B.P. °C/mmHg	M.P. °C	Ti	
			Found	Calcd.
(*n*-$C_3H_7O)_4Ti$	137–40/3a)		16.9%	17.0%
(*iso*-$C_3H_7O)_4Ti$	97– 8/8	15	16.8	17.0
(*sec*-$C_4H_9O)_4Ti$	107– 9/3b)		14.2	14.1
(*tert*-$C_4H_9O)_4Ti$	85– 7/3c)		14.2	14.1
(*n*-$C_5H_{11}O)_4Ti$	182– 3/3d)		12.3	12.1
(*tert*-$C_5H_{11}O)_4Ti$	129–30/2e)		12.2	12.1
$(C_6H_{11}O)_4Ti$	220– 5/3f)	43	10.6	10.8
(*n*-$C_7H_{15}O)_4Ti$	215–20/1g)		9.47	9.42

a) 170/3[5], 171/14[7], b) 90–2/0.5–1[5], c) 62–3/1[5], 102/1[7], d) 175/0.8[5], 158/0.1[8], 211/11[7], e) 98/0.1[9], f) 130.5–2.0/1[10], g) 213/0.4[9], 201/0.1[9]264–5/15[11].

***n*-Propyl Titanate.**—A mixture of ethyl titanate (22.8 g, 0.1 mol.) and *n*-propanol 30.1 g, 0.5 mol.) was heated to remove the ethanol produced during the reaction. After the theoretical quantity of ethanol was recovered, the residue was distilled under reduced pressure. *n*-Propyl titanate was obtained in 75% yield.

***tert*-Butyl Titanate.**—It was impossible to prepare this compound by reacting titanium tetrachloride with *tert*-butanol or sodium-*tert*-butoxide. The manipulation of the transesterification reaction between ethyl titanate and *tert*-butanol was somewhat complicated, because the separation of ethanol produced and *tert*-butanol by fractional distillation was difficult.

tert-Butyl titanate was obtained from ethyl titanate (22.8 g, 0.1 mol.) and *tert*-butanol (37.0 g, 0.5 mol.) following the method as in the case of *n*-propyl titanate. After ethanol and *tert*-butanol were distilled off, the residue was treated with *tert*-butanol (7.4 g, 0.1 mol.). This procedure was repeated ten times to complete reaction, *tert*-butyl titanate being obtained by distillation under reduced pressure in 52% yield.

Results and Discussion

There are eighteen sets of densities and refractive indices of organic titanates and chlorotitanates which were observed at the same temperature as shown in Table III. From these data, molecular refraction of each compound was calculated. The sum of Denbigh's bond refraction[15] for C–C, C–H, C–O was reduced from the molecular refraction of the compound, the remainder being divided by the number of Ti–O bonds in a molecule, and the bond refraction for Ti–O was obtained. As the bond refraction for Ti–Cl has not appeared in the literature, it was calculated, using

TABLE II

MOLECULAR REFRACTIONS OF THE PREPARED TITANATES

Compd.	Dens. d_4^t	Refr. Ind. n_D^t	Temp. °C	Mol. Refr. (ml./mol.)		
				Obs.	Calcd.	Error
(*n*-$C_3H_7O)_4Ti$	1.0358a)	1.4907a)	35	79.41	79.68	−0.34%
(*iso*-$C_3H_7O)_4Ti$	0.9711	1.4678	20	81.34	79.68	+2.0
(*sec*-$C_4H_9O)_4Ti$	0.9463a)	1.4587a)	35	98.27	98.20	+0.07
(*tert*-$C_4H_9O)_4Ti$	0.8917a)	1.4368a)	20	99.96	98.20	+1.8
(*n*-$C_5H_{11}O)_4Ti$	0.9703	1.4813	35	116.34	116.56	−0.18
(*tert*-$C_5H_{11}O)_4Ti$	0.9118	1.4543	20	117.82	116.56	+1.1
$(C_6H_{11}O)_4Ti$	1.0589b)	1.5155b)	35	126.69	126.72	−0.12
(*n*-$C_7H_{15}O)_4Ti$	0.9430a)	1.4830a)	20	154.05	153.76	+0.20

a) Literature is shown in Table III.
b) Supercooled Liquid.

4) O. V. Nogina, and A. B. Belyavskii, *Akad. Nauk S. S. S. R., Inst. Org. Khim., Sintezy Org. Soedinenii, Sbornik*, **2**, 164 (1952), *Chem. Abst.*, **48**, 567c (1954).
5) R. J. Speer, *J. Org. Chem.*, **14**, 655 (1949).
6) D. C. Bradley, F. M. Abd-el Halim and W. Wardlaw, *J. Chem. Soc.*, **1950**, 3450.
7) N. M. Cullinane. S. J, Chard, G. F. Price, B. B. Millward and G. Langlois, *J. Appl. Chem.*, **1**, 400 (1951).
8) D. C. Bradley, R. C. Mehrotra and W. Wardlaw, *J. Chem. Soc.*, **1952**, 2027.
9) D. C. Bradley, R. C. Mehrotra, J. D. Swanick and W. Wardlaw, ibid., **1953**, 2025.
10) O. A. Nogina, R. Kh. Freidlina and A. N. Nesmeyanov, *Izvest. Akad. Nauk S. S. S. R., Otdel. Khim. Nauk*, **1950**, 32, *Chem. Abst.*, **44**, 8854 i (1950).
11) B. A Arbuzov and T. G. Shavsha, *Doklady Akad. Nauk S. S. S. R.*, **68**, 859 (1949), *Chem. Abst.*, **44**, 885 e (1950).

TABLE III

CALCULATED BOND REFRACTIONS FOR Ti-O FROM THE DATA IN LITERATURE

Compounds	Dens. d_4^t	Refr. Ind. n_D^t	Temp. °C	Bond Refr. for Ti-O ml./mol.	Lit.
$(C_2H_5O)_4Ti$	1.1071	1.5051	35	4.07	5)
$(n\text{-}C_3H_7O)_4Ti$	0.9970	1.4803	35	4.42a)	5)
$(n\text{-}C_4H_9O)_4Ti$	1.0051	1.4925	20	4.11	12)
$(n\text{-}C_4H_9O)_4Ti$	0.9927	1.4863	35	4.16	5)
$(sec\text{-}C_4H_9O)_4Ti$	0.9196	1.4550	35	4.65a)	5)
$(tert\text{-}C_4H_9O)_4Ti$	0.8893	1.4436	20	4.93a)	5)
$(n\text{-}C_6H_{13}O)_4Ti$	0.9573	1.4830	20	4.03	12)
$(n\text{-}C_7H_{15}O)_4Ti$	0.8963	1.4810	20	4.59a)	11)
$(n\text{-}C_8H_{17}O)_4Ti$	0.9339	1.4810	20	4.09	12)
$(n\text{-}C_9H_{19}O)_4Ti$	0.9241	1.4785	20	3.97	12)
$(CH_3OC_2H_4O)_4Ti$	1.1910	1.5077	18	4.17	13)
$(C_2H_5OC_2H_4O)_4Ti$	1.1106	1.4920	21	4.17	13)
$[(CH_3)_3SiO]_4Ti$	0.9078	1.4278	20	4.02	1)
$(n\text{-}C_4H_9O)_3TiCl$	1.0985	1.5169	20	4.16	14)
$(C_2H_5OC_2H_4O)_3TiCl$	1.2035	1.5178	20	4.03	14)
$(iso\text{-}C_4H_9O)_3TiCl$	1.1043	1.5158	20	3.97	14)
$(iso\text{-}C_5H_{11}O)_3TiCl$	1.0600	1.5092	20	4.13	14)
$(n\text{-}C_6H_{13}O)_3TiCl$	1.0039	1.5050	20	5.25a)	14)
				Mean 4.08	

a) Except for the calculation of the mean value.

the values of titanium tetrachloride, d_4^{25}·[1] 1.7344 and n_D^{20}·[1] 1.6085, obtaining 9.46 ml./mol.[16] This value was used throughout the present investigation.

Eighteen bond refraction values for Ti–O were thus obtained, but as five values among them were far apart from the other thirteen values, the mean value was taken for all except these five values. The average bond refraction for Ti–O was obtained as 4.08 ml./mol.

Then, *n*-propyl, *sec*-butyl, *tert*-butyl, *n*-heptyl, *iso*-propyl, *n*-pentyl, *tert*-pentyl and cyclohexyl titanate were prepared, purified, and their densities and refractive indices were measured. In the calculation of the mean value of bond refractions for Ti–O the first four titanates were excepted.

Densities and refractive indices measured at the same temperature of the last four titanates have not been reported in the literature.

Molecular refractions obtained from these data of the titanates prepared were compared with the calculated ones using the bond refraction for Ti–O, 4.08 ml./mol. Both were in good agreement except for *iso*-propyl, *tert*-butyl, and *tert*-pentyl titanate. Thus the value 4.08 ml./mol. for Ti–O, was justified by these considerations.

The errors of molecular refractions of *iso*-propyl, *tert*-butyl, and *tert*-pentyl titanate amount to +2.0, +1.8, +1.1 %, respectively. According to Denbigh, the observed molecular refractions of many compounds agree with the calculated ones in a range of ±0.7 % error.

Considering this fact, the observed molecular refractions of these three titanates seem to be considarably large. The purity of these titanates was sufficient, viewed from their titanium contents. Particularly, *iso*-propyl titanate was obtained in a crystalline form by leaving the liquid standing for a long time. The cause of the observed greater molecular refractions of these titanates may be attributed to their greater hygroscopicity. Actually, refractive indices of these compounds gradually change greater during the measurement using Abbe's refractometer.

In the course of the preparation of *tert*-butyl and *tert*-butyl titanate, an interesting fact was observed. It has been indicated by us[17] that the transesterification reaction between ethyl silicate and *tert*-butanol

12) B. A. Arbuzov and T. G. Lsaeva, *Zhur. Obshchei Khim. S. S. S. R.*, **22**, 566 (1952), *Chem. Abst.*, **47**, 2684 g (1953).

13) A. N. Nesmeyanov, R. Kh. Freidlina and O. V. Nogina, *Izvest. Akad. Nauk S. S. S. R., Otdel. Khim. Nauk*, **1951**, 518, *Chem. Abst.*, **46**, 7038 i (1952).

14) O. V. Nogina, R. Kh. Freidlina and A. N. Nesmeyanov, *Izvest. Akad. Nauk S. S. S. R., Otdel. Khim. Nauk*, **1952**, 74, *Chem. Abst.*, **47**, 1583 f (1953).

15) K. G. Denbigh, *Trans. Farad. Soc.*, **36**, 936 (1940).

16) "Landolt-Börnstein Physiknlisch-Chemischen Tabellen". 5 Aufl. Zweiter Frgänzba. Zweiter Teil. Verlag von, Julius Springer Berlin (1931), S. 815.

17) Presented at the Annual Meeting of the Chem. Soc. of Japan in 1955.

or *tert*-pentanol proceeds incompletely as shown in the following equation (2)

$$(C_2H_5O)_4Si + 2\,tert\text{-}C_4H_9OH = (C_2H_5O)_2(tert\text{-}C_4H_9O)_2Si + 2\,C_2H_5OH. \quad (2)$$

However, when ethyl titanate was used in place of ethyl silicate, the transesterification reaction proceeded almost completely, and the titanates tetrasubstituted by *tert*-alkyl groups were obtained. It seems that titanium, the central metallic atom of the titanate, is large enough to diminish the steric effect of *tert*-alkoxy groups, which caused the incomplete transesterification reaction in the case of silicium.

The author wishes to express his sincere gratitute to Dr. M. Tachimori, Dr. S. Tsuruta and Dr. H. Kitagawa for their interest kindly extended to him throughout the present investigation.

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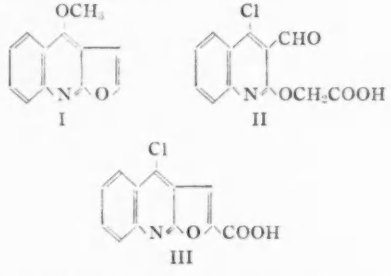
Synthetic Studies of Dictamnine, an Alkaloid of Skimmia repens, Nakai. II. The Synthesis of Dictamnine

By Tadashi SATO and Masaki OHTA

(Received October 20, 1956)

As an approach to the synthesis of dictamnine (I), some experiments were reported in our previous paper[1]. Recently the synthesis of dictamnine was reported by Tuppy et al.[2], but its details have not yet been known. In this communication we describe the synthesis of dictamnine accomplished independently in our laboratory.

As previously reported, we synthesized the aldehyde II, and tried to cyclize it to the furoquinoline derivative III. Numerous attempts, however, to effect the cyclization of II to III were unsuccessful.



I II III

In their study of synthesizing dictamnine, Grundon et al. obtained 2,4-dichloro-3-(2-chloroethyl)-quinoline (IV)[3]. In our previous paper we reported that only the 2-chlorine atom of the 2,4-dichloroquinoline derivatives could selectively be replaced by a hydroxyl group by treatment with glacial acetic acid. On applying the same method to the trichloro derivative IV, a mixture of two substances was obtained, and these were considered as the 2-hydroxyquinoline derivative V and the dihydrofuroquinoline derivative VII, from their ultraviolet spectroscopic characteristics. The spectra of V (Fig. 1) were similar to those of the 2-hydroxyquinoline derivatives in neutral as well as in acid solution. As noted in our previous paper, the absorption

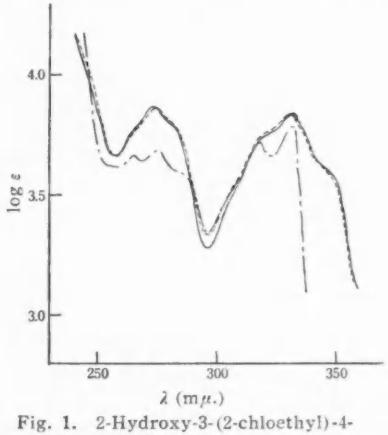
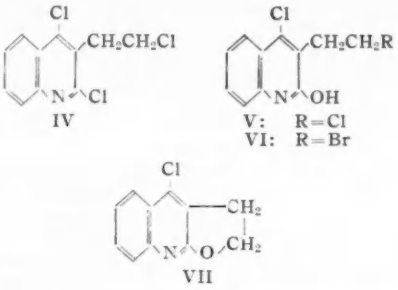


Fig. 1. 2-Hydroxy-3-(2-chloethyl)-4-chloroquinoline (V)
neutral ---- acid
—— alkaline

1) T. Sato and M. Ohta, This Bulletin, **29**, 817 (1956).
2) H. Tuppy, et al., *Angew. Chem.*, **68**, 388 (1956).
3) M. F. Grundon, et al., *J. Chem. Soc.*, **1955**, 4284.



maximum (275 mμ) of 2-hydroxyquinoline derivatives flattens generally in alkaline solution, but the spectrum of V in alkaline solution becomes identical with that of VII (Fig. 2) owing to cyclization, and the

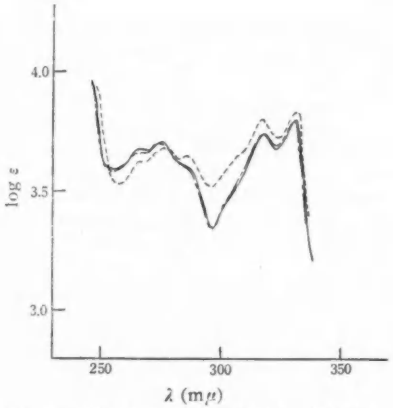
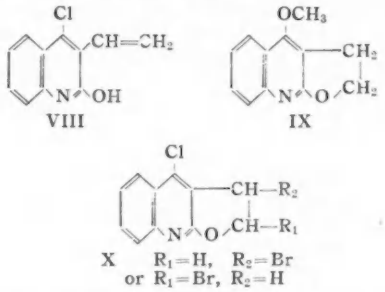


Fig. 2. 4-Chloro-2,3-dihydrofuro[2,3-b] quinoline (VII)
——— neutral ---- acid
— — — alkali

characteristics described above could not be observed. The different spectroscopic characteristics of VII from those of the 2-hydroxyquinoline derivatives exclude the possibility that VII might be the vinyl derivative such as VIII. When VII was heated with hydrogen bromide, the bromo derivative VI was obtained, whose spectroscopic characteristics were very similar to those of the chloro derivative V.

Though it was difficult to separate V and VII, VII was obtained readily in pure state when the crude product was treated with methanolic sodium hydroxide. The methoxy derivative IX was obtained from the chloro derivative VII by treatment with sodium methylate at 100°. Our methoxy derivative melted at 92° (sintering at 82–3°), and gave the picrate having a m.p. of 187°, while the m.p's of the specimens from a natural source are 96–7° and 183°, respectively[4].



The dehydrogenation of the dihydrofuroquinoline derivatives VII and IX to the corresponding furoquinoline derivatives was not effected by palladium-charcoal in boiling diphenyl ether.

In order to obtain the bromo derivative X, we treated the chloro derivative VII with bromine, but the substance thus obtained was the perbromide having an empirical formula of $C_{11}H_5NOClBr$.

The desired bromo derivative X was obtained when the chlorodihydrofuroquinoline derivative VII was refluxed with N-bromosuccinimide in carbon tetrachloride in the presence of a small amount of benzoyl peroxide, and the solvent was removed under a reduced pressure though the position of the bromine atom was uncertain. When carbon tetrachloride was not removed, another substance decomposing at 92° was obtained. Although its structure has not yet been determined, it gave the bromo derivative X when treated with pyridine. The methoxydihydrofuroquinoline derivative IX gave no well-defined product with N-bromosuccinimide. The desired dictamnine (I) was obtained by the reaction of the bromo derivative X with sodium methylate in a sealed tube at 100°.

The synthetic dictamnine was shown to be identical with the natural specimen by their melting points, mixed melting points and melting points of their picrates.

Experimental

Absorption spectra.—All spectra were determined in the same way as reported in our previous paper.

2,4-Dichloro-3-(2-chloroethyl)-quinoline (IV).—It was prepared by the Grundon's method[3] with slight modifications. Methyl cellosolve was used as a starting material instead of cellosolve;

4) T. Ohta, et al., *J. Pharm. Soc. Japan.* **74**, 708(1954).

p-toluenesulfonyl chloride instead of benzenesulfonyl chloride was used for preparing the malonic acid derivative; and the amount of diphenyl ether in the condensation of aniline with the malonic acid derivative was minimized. Namely, 40 g. of diethyl (2-methoxyethyl)-malonate and 12 g. of aniline were refluxed in 60 g. of diphenyl ether for three hours. Thus, on cooling the reaction mixture, fairly pure 4-hydroxy-2,3-dihydrofuro[3,2-*c*]quinoline, m.p. 275° (decomp.), was obtained. Yield 19 g. The crude product was treated with phosphorus oxychloride and the trichloro derivative IV thus obtained was used without purification in the next step.

2-Hydroxy-3-(2-chloroethyl)-4-chloroquinoline (V).—A solution of 17 g. of the trichloro derivative IV in 100 cc. of glacial acetic acid was heated under a reflux for three hours, after which about 200 cc. of water was added. The product was collected, and washed with water, m.p. 130—170°. Yield 15 g. Repeated recrystallization from dilute methanol or dilute acetic acid raised the m.p. to 190°.

Anal. Found: C, 54.62; H, 3.94; N, 5.47. Calcd. for $C_{11}H_9NOCl_2$: C, 54.55; H, 3.72; N, 5.78%.

4-Chloro-2,3-dihydrofuro[2,3-*b*]quinoline (VII).—In the other recrystallization process of the crude product described above, the cyclized derivative VII, m.p. 113°, was obtained instead of the open-chain derivative V.

Anal. Found: C, 63.92; H, 4.09; N, 7.10. Calcd. for $C_{11}H_8NOCl$: C, 64.08; H, 3.88; N, 6.79%.

VII was more conveniently obtained as follows: A mixture of 15 g. of the crude product melting at 130—170°, resulting from the reaction of the trichloro derivative IV with glacial acetic acid, and 100 cc. of methanol solution containing 5 g. of sodium hydroxide was heated under a reflux for two hours, and then about 200 cc. of water was added. The dihydrofuroquinoline derivative VII thus obtained was sufficiently pure for the next step, m.p. 111°. Yield 8 g.

2-Hydroxy-3-(2-bromoethyl)-4-chloroquinoline (VI).—To a solution of 0.1 g. of the dihydrofuroquinoline derivative VII in 1.5 cc. of glacial acetic acid was added 1 cc. of 48% aqueous hydrobromic acid solution and heated under a reflux for fifteen minutes. On adding water, white needles were obtained. Recrystallization from dilute acetic acid gave an analytical sample, m.p. 190°.

Anal. Found: C, 46.10; H, 3.44; N, 5.26. Calcd. for $C_{11}H_9NOBrCl$: C, 45.99; H, 3.14; N, 4.88%.

4-Methoxy-2,3-dihydrofuro[2,3-*b*]quinoline (IX).—To a solution of 0.4 g. of the chloro derivative VII in 3 cc. of methanol, 5 cc. of sodium methylate solution containing 0.15 g. of sodium, was added and heated in a sealed tube at 100° for three hours. After being set aside overnight the sodium chloride was filtered off and the filtrate was concentrated to about 3 cc. under a reduced pressure. On addition of water, white crystals were obtained. Recrystallization from dilute methanol afforded white needles, m.p. 92° (sintering at 82—3°).

Anal. Found: C, 70.32; H, 5.67; N, 7.14. Calcd. for $C_{12}H_{11}NO_2$: C, 71.64; H, 5.47; N, 6.96%.

The picrate was prepared in ether and was recrystallized from methanol, m.p. 187°.

Anal. Found: N, 13.12. Calcd. for $C_{18}H_{14}N_4O_9$: N, 13.02%.

4-Chloro-2,3-dihydrofuro[2,3-*b*]quinoline perbromide.—To a solution of 0.3 g. of VII in 10 cc. of carbon tetrachloride was added 0.23 g. of bromine dissolved in 1 cc. of carbon tetrachloride within twenty minutes with stirring at room temperature. After being stirred for one additional hour, the yellow crystals were collected and reprecipitated from methanol solution with water at room temperature, m.p. 122° (decomp.).

Anal. Found: C, 46.69; H, 3.17; N, 5.09. Calcd. for $C_{11}H_8NOClBr$: C, 46.15; H, 2.80; N, 4.89%.

2- or 3-bromo-4-chloro-2,3-dihydrofuro[2,3-*b*]quinoline (X).—A mixture of 1 g. of dihydrofuroquinoline derivative VII, 1 g. of N-bromosuccinimide and 0.1 g. of benzoyl peroxide in 50 cc. of carbon tetrachloride was heated under a reflux for forty-five minutes. Afrer the succinimide was filtered off, the solution was concentrated to about 15 cc. On cooling, the bromo derivative X was obtained, m.p. 120°.

Anal. Found: C, 46.08; H, 2.78; N, 5.26. Calcd. for $C_{11}H_7NOClBr$: C, 46.31; H, 2.46; N, 4.91%.

When carbon tetrachloride was not removed, a substance decomposing at 92° was obtained. When its pyridine solution was diluted with water, the bromo derivative X crystallized in white needles, but its further recrystallization from pyridine-water was unsuccessful. Recrystallization of the substance decomposing at 92° or of the bromo derivative X from dilute methanol gave a substance with a m.p. of 113°, whose structure could not be determined.

Dictamnine (I).—To a solution of 0.15 g. of the bromo derivative X in 2 cc. of methanol was added 2.5 cc. of sodium methylate solution containing 0.075 g. of sodium. The mixture was heated in a sealed tube at 100° for two hours and allowed to stand overnight. After the inorganic salts were filtered off and methanol was partly evaporated, water was added. The dictamnine thus obtained was fairly pure, melting at 130—1°, (natural product; m.p. 132—3°)[5]; a mixed melting point with natural dictamnine was 130—1°.

Anal. Found. C, 72.11; H, 4.72; N, 7.14. Calcd. for $C_{12}H_9NO_2$: C, 72.35; H, 4.55; N, 7.03%.

The melting point of its picrate obtained in the same way as described in the literature[5] also coincided with that from natural source (m.p. 163°).

5) Y. Asahina, T. Ohta and M. Inubuse, *Ber.*, **63**, 2045 (1930).

We wish to express our grateful appreciation to Professor T. Ohta of the Tokyo College of Pharmacy who kindly provided us with the natural dictamnine for identification, and to Mr. A. Kondo for microanalyses.

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Hydrogen Bonding Effect on the Fluorescence of π-Electron System. III. Fluorescence Quenching of Some Nitrogen Heterocycles Caused by Hydrogen Bond Formation*

By Noboru MATAGA and Shizuyo TSUNO

(Received April 16, 1957)

In II[1] of this series, we have interpreted the inner quenching of fluorescence of some nitrogen heterocycles as due to the interaction between *n*- and π-electrons, on the basis of the fact that the enhancements of fluorescence yields of these molecules caused by hydrogen bonding is greater, the stronger the donating powers of proton donors used. The fluorescence yields of acridine hydrogen bonded with ethanol, β-phenylethylalcohol and benzylalcohol are almost the same. We can observe, however, no enhancement of fluorescence yield, when acridine is hydrogen bonded with phenol which is capable of making hydrogen bond intimately connected with π-electron system in contrast to molecules such as benzylalcohol and β-phenylethylalcohol. Actually, we have observed a remarkable fluorescence quenching by phenol when 3,6-diaminoacridine (DAA) and 3,6-bisdimethylaminoacridine (BDAA) which are able to fluoresce fairly well even in non-polar solvent, are used as fluorescer and proton acceptor.

These facts indicate that the interaction between π-electron systems via the hydrogen bond may lead to the non-radiative degradation of excited state. To elucidate the problem further, we have used various proton donors such as naphthols, phenol, aniline and pyrrole, the hydrogen bonding by which are intimately related with π-electron system.

For all of these donors, strong or moderate decrease of fluorescence intensity was observed. Moreover, the effect of viscosity on the quenching was studied by changing the hydrocarbon solvents from *n*-hexane to liquid paraffin, and no viscosity dependence of quenching was observed. In the present paper, we will report these facts with some discussions on the mechanism of fluorescence quenching caused by the interaction between π-electron systems of fluorescer and of quencher molecules.

Experimental

Apparatus.—Absorption spectra were measured with Beckman spectrophotometer model DU. Fluorometer was the same as described previously[2]. A high pressure mercury lamp with appropriate filters was used as an exciting light source. Matsuda color filter UV-DI was used for all samples as an exciting light filter.

For the fluorescent light, the light filter used for acridine was the same as before[2], and for DAA and BDAA, Matsuda color filter VY—I was used.

Reagents.—Acridine, DAA[3] and BDAA[3] were the same sample as used previously. *n*-Hexane and benzene were the same as reported before[1]. Cyclohexane and decaline were shaken with fuming sulfuric acid diluted with conc. sulfuric acid and distilled carefully. Liquid paraffin was purified through the column of activated alumina for removing the fluorescing impurity. Extra pure grade phenol was distilled carefully before use. Aniline was purified by the method of Keyes and Hildebrand[4], and stored in vacuo. Commercial pyrrole was distilled carefully and stored in vacuo.

Experimental Results

A. Change of Absorption Spectrum by

* I and II of this series: N. Mataga, Y. Kaifu and M. Koizumi, This Bulletin, **29**, 115 (1956), and N. Mataga and S. Tsuno, ibid., **30**, 368 (1957).

1) N. Mataga and S. Tsuno, loc. cit.

2) N. Mataga, Y. Kaifu and M. Koizumi, This Bulletin **29**, 373 (1956).

3) N. Mataga, ibid., **30**, 375 (1957).

4) D. B. Keyes and J. H. Hildebrand, *J. Am. Chem. Soc.*, **39**, 2126 (1917).

Hydrogen Bonding.—According to Zanker[5], the lowest excited singlet states of DAA and BDAA are ^1L_a as it is the case for acridine. When the proton donors are added to the solutions of these nitrogen heterocycles in non-polar solvents, the absorption spectra show characteristic changes due to the hydrogen bonding equilibrium. In all these molecules, hydrogen bonding induces the red shift of ^1L_a band. Some examples of these spectral changes caused by hydrogen bonding are shown in Figs. 1 and 2.

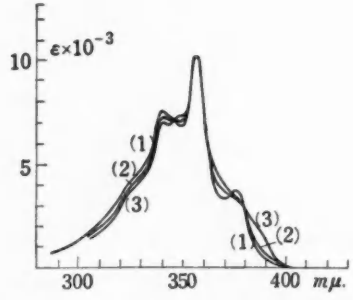

Fig. 1. Change of absorption spectrum of acridine in benzene by added phenol. Concentration of acridine: 5.00×10^{-5} mole/l. Concentration of phenol: (1) 0, (2) 7.12×10^{-3} mole/l., (3) 3.56×10^{-2} mole/l.

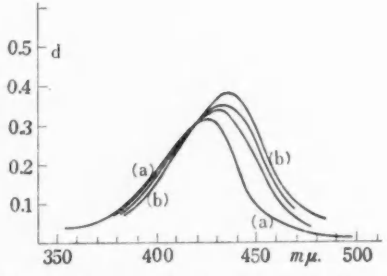

Fig. 2. Change of absorption spectrum of BDAA in benzene by added phenol. Concentration of BDAA: 1.36×10^{-5} mole/l. Cell length: 1 cm. Concentration of added phenol: (a) 0, (b) 9.4×10^{-3} mole/l.

5) V. Zanker, *Z. physikal. Chem.*, N. F., **2**, 52 (1954).

* The concentration ranges of donors used for the calculation of equilibrium constants, are as follows.
phenol and naphthols: $10^{-4}\sim10^{-2}$ mol./l., where nearly 100% monomer exists. [a)]
(a) R. Mecke, *Disc. Faraday Soc.*, No. **9**, 161 (1950).
aniline: $10^{-2}\sim10^{-1}$ mol./l., where nearly 100% monomer exists.[b)]
pyrrole: $5\times10^{-2}\sim4\times10^{-1}$ mol./l., where 90~70% monomer exists.[b)]
(b) N. Fuson, M. L. Josien, R. L. Powell and E. Utterback, *J. Chem. Phys.*, **20**, 145 (1952).

From these spectral changes, we can evaluate the equilibrium constant of hydrogen bond formation in the ground state, using Eq. (1)[1)]*.

$$\frac{1-(d_0/d)}{[D]} = -K+\left(\frac{\varepsilon_c}{\varepsilon_a}\right)K(d_0/d) \qquad (1)$$

Some examples of analyses using this formula were given in Figs. 3 and 4.

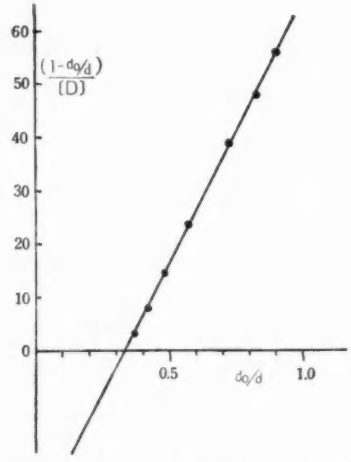

Fig. 3. $(1-d_0/d)/[D]\sim d_0/d$ relation for acridine-phenol-benzene system at 386 mμ.

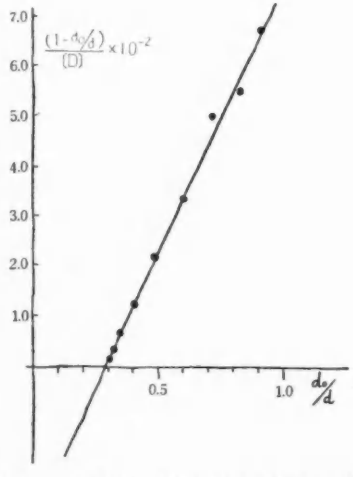

Fig. 4. $(1-d_0/d)/[D]\sim d_0/d$ relation for BDAA-phenol-benzene system at 448 mμ.

B. Effect of Hydrogen Bonding on the Fluorescence Intensity.—Acridine is nonfluorscent in non-polar solvent, and no enhancement of fluorescence intensity was

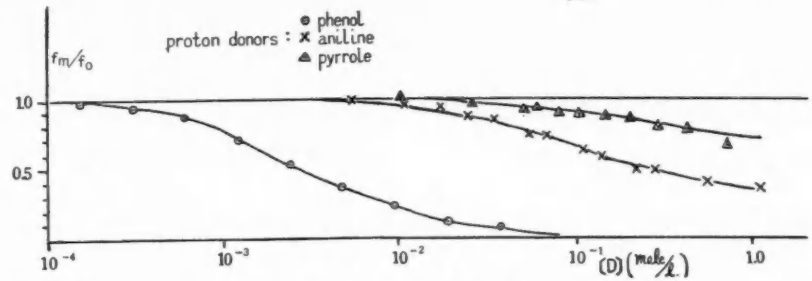

Fig. 5. Changes of relative fluorescence intensity of BDAA in benzene by added various proton donors.

observed when it is hydrogen bonded with phenol.

DAA and BDAA are fluorescent even in a non-polar solvent, and decrease in fluorescence intensity is observed, when various proton donors such as phenol, naphthols, aniline and pyrrole are added in the solution. Some examples of quenching curves for BDAA in benzene are given in Fig. 5.

The observed changes of fluorescence intensity may most probably be attributed to the fact that the free and the hydrogen bonded molecules have different efficiency of fluorescence and that the concentration of these molecular species are determined by association equilibrium. Then, from these changes of fluorescence intensity, we can evaluate the equilibrium constant of hydrogen bond formation and the ratio of the quantum yields of fluorescences of hydrogen bonded and free molecules, using the following formulas, (2) and (3)[1]*.

$$\frac{1-(f_0/f_m)}{[D]} = -K+\alpha\left(\frac{\varepsilon_c}{\varepsilon_a}\right)K(f_0/f_m) \qquad (2)$$

$$\frac{1-(f_0/f_m)(d_m/d_0)}{[D]} = -K'+\alpha\left(\frac{d_m}{d_0}\right)K'(f_0/f_m) \qquad (3)$$

Owing to a very small difference of extinction coefficients of free and hydrogen bonded molecules at the wave length of exciting light, (2) and (3) practically coincide in their forms and it has been established that the experimental results can be reproduced by either Eq. (2) or (3) satisfactorily for all cases studied, the equilibrium constants evaluated by Eqs. (2) and (3) being paractically the same. Some examples of these analysis are given in Figs. 6 and 7. Further, the equilibrium constant obtained in this way from fluorescence intensity measurement agrees with that evaluated from the change in absorption spectrum.

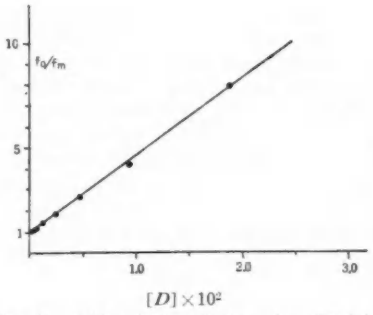

Fig. 6. $f_0/f_m \sim [D]$ relation for BDAA-phenol-benzene system[a]
(a) Conditions, $\alpha=0$ in Eq. (2) or $\alpha=0$, $d_m=d_0$ in Eq. (3), are satisfied in this case.

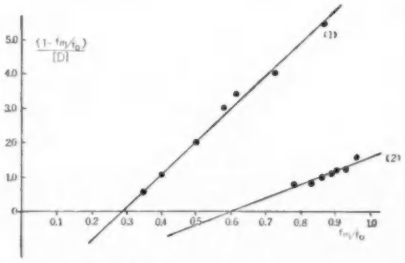

Fig. 7. $\frac{(1-f_m/f_0)}{[D]} \sim f_m/f_0$ relations for BDAA-aniline-benzene (1) and BDAA pyrrole-benzene (2) systems[b].
(b) (Conditions $d_m=d_0$ in Eq. (3) or $\varepsilon_c=\varepsilon_a$ in Eq. (2), are satisfied in these cases.)

Therefore, the association equilibrium in the excited state is practically the same as that in the ground state.

C. Effect of Solvent Viscosity on the Fluorescence Quenching.—If the diffusion controlled dynamical processes in the excited state such as the energy dissipation due to dissociation of hydrogen bond or

TABLE I

acceptor	donor	solvent	T	K_T	ϕ_c/ϕ_0
acridine	phenol	benzene	287	32	
BDAA	α-naphthol	〃	306	5.7×10^2	~0
	β-naphthol	〃	304	2.9×10^2	~0
	phenol	〃	300	3.7×10^2	~0
	aniline	〃	300	9.6	0.29
	pyrrole	〃	300	3.8	0.59
	phenol	n-hexane	294	1.3×10^3	0.02
	〃	cyclohexane	298	1.4×10^3	0.01
	〃	decalin	298	0.9×10^3	0.06
	〃	liquid paraffin	297	1.4×10^3	~0
DAA	phenol	benzene	286	6.3×10^2	~0
	aniline	〃	287	20	0.29

rotation around the hydrogen bond[6] are concerned with the non-radiative degradation of excited state, the ratio of the fluorescence yields of hydrogen bonded and free molecules, ϕ/ϕ_0, may show some dependence on the solvent viscosity. From such a viewpoint, we have examined the effect of solvent viscosity on the fluorescence quenching of BDAA by phenol, changing the solvent from n-hexane to liquid paraffin. The results obtained are shown in Fig. 8 and Table I.

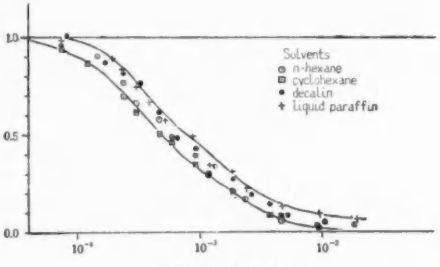

Fig. 8. Changes in relative fluorescence intensity of BDAA in various solvents of different viscosities by added phenol.

No viscosity dependence has been observed and the fluorescence is almost completely quenched by hydrogen bonding with phenol, in all the solvents examined. Therefore, no dynamical mechanism, in which the molecule moves as a whole, may prevail in the quenching of the present type.

Discussion

The results obtained are collected in the Table I. When hydrogen bond is isolated from π-electron system of donor molecule by σ-bond such as the case for benzylalcohol and β-phenylethylalcohol, the enhancement of fluorescence yield by the mechanism described previously[1], occurs. However, when hydrogen bond is intimately related with π-electron system, i. e. the conjugation of hydrogen bond with π-electron system is possible, such as the case for phenol, naphthols, aniline and pyrrole, the decrease in fluorescence yield was observed. Moreover, from the independence of quenching of the viscosity of the solvent, it is clear that any dynamical process in the excited state, in which the quencher molecule moves as a whole, does not concern the degradation of excited state. Therefore, it is most likely that the cause of non-radiative degradation may be ascribed to the interaction between π-electron systems via the hydrogen bond, i. e. the delocalization of π-electron through the hydrogen bond, in the excited state. An analogous mechanism in which the charge transfer interaction between excited anthracene and solvent molecule leads to the fluoresecnce quenching, was proposed by Bowen and West[7]. From the stand-point mentioned above, a merely electrostatic model of the hydrogen bond may not be appropriate.

A charge transfer model of hydrogen bond using molecular orbital and perturbation theory, was proposed by Nukasawa, et al.[8] and supported by Tsubomura on the basis of his detailed calculation[9] for the system O—H···O and also from the result of his infrared measurement[10]. From the viewpoint of the quenching phenomenon with which the present paper

6) G. Oster and Y. Nishijima, *J. Am. Chem. Soc.*, **78**, 1581 (1956).

7) E. J. Bowen and K. West, *J. Chem. Soc.*, **1955**, 4394.

8) K. Nukasawa, J. Tanaka and S. Nagakura, *J. Phys. Soc. Japan*, **8**, 792 (1953).

9) H. Tsubomura, This Bulletin, **27**, 445 (1954).

10) H. Tsubomura, *J. Chem. Phys.*, **23**, 2130 (1955); ibid., **24**, 927 (1956).

is concerned, the charge transfer model of hydrogen bond is more favored than the merely electrostatic one, especially in the excited state.

A similar phenomenon was also observed for the fluorescence quenching of naphthols and naphthylamines due to hydrogen bonding with pyridine, and this type of non-radiative degradation of the excited state due to the delocalization of π-electron through the hydrogen bond, may form a group in the quenching phenomena by complex formation.

Summary

1. Hydrogen bonding effect on the fluorescence of acridine, 3,6-diaminoacridine and 3,6-bisdimethylaminoacridine was studied in non-polar solvent added with proton donors such as phenol, naphthols, aniline and pyrrole.

2. Their fluorescence yields are decreased when hydrogen bonded with these proton donors, in which the hydrogen bond is capable of conjugation with π-electron system, in contrast to the case of donors such as benzylalcohol and β-phenylethylalcohol in which the hydrogen bond is isolated by σ-bond from π-electron system, and hydrogen bonding with which leads to the enhancement of fluorescence yield.

3. No viscosity dependence of quenching has been observed. From this fact participation of diffussion controlled dynamical process in the non-radiative degradation of excited state is unlikely.

4. The cause of non-radiative degradation of excited state by hydrogen bonding was ascribed to the delocalization of π-electrons through the hydrogen bond, especially in the excited state.

The authors express their gratitude to Professor M. Koizumi of Tohoku University and Professor R. Fujishiro for their interest in the present work. Their cordial thanks are due to Mr. H. Tsubomura of Tokyo University for his illuminating discussions.

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Spectrochemical Study of Microscopic Crystals. XV[1]*. Structure and Light Absorption of Bis-(dimethylglyoximato) gold(III) Dichloro-aurate(I)*[2]

By Shoichiro YAMADA and Ryutaro TSUCHIDA

(Received April 17, 1957)

In the former publications[3] the present authors reported on those compounds which display enormously deep colors owing to possible metal-to-metal interaction. A recent study indicated that bis-(dimethylglyoximato)-gold(III) dichloro-aurate(I), $[Au(dgH)_2][AuCl_2]$[4], would be a compound of a similar sort. Rundle[5] examined with X-ray the crystal structure of the compound, showing that in the crystal of the compound the linear dichloro-aurate(I), $[AuCl_2]^-$, and planar bis-(dimethylgyloximato)-gold(III) ions, $[Au(dgH)_2]^+$, are arranged parallel to each other, with the gold(I) and the gold(III) ions disposed at an equal distance along the *c*-axis of the crystal. Sinec the distance between the gold(I) and the gold(III) ions was found to be comparatively short, he assumed the existence of the direct interaction between the gold(I) and the gold(III) ions. As our former reports revealed that those compounds involving direct interaction between metallic ions showed an extraordinary type of dichroism, it was thought that it would be significant to examine dichroism of bis-(dimethylglyoximato)-gold(III) dichloro-aurate(I) with reference to the relationships on the dichroism which were formerly derived.

1) Part XIV of this series, S. Yamada, H. Nakamura and R. Tsuchida, This Bulletin, **30**, 647 (1957).

2) R. Tsuchida and S. Yamada, Presented before the Annual Meeting of Japan Chemical Society, Tokyo, April 6th, 1957.

3) S. Yamada and R. Tsuchida, This Bulletin, **27**, 156 (1954); *J. Am. Chem. Soc.*, **73**, 1579 (1951); ibid., **75**, 6351 (1953), etc.

4) The notation, dgH_2, represents a molecule of dimethylglyoxime.

In this work we have quantitatively determined the dichroism of the above-mentioned compound in the visible and the ultra-violet. The result has been found to disagree with the hypothesis of the existence of the metal-to-metal interaction in the present compound.

Experimental

Material.—The compound, $[Au(dgH)_2][AuCl_2]$ was prepared in brown needles according to the method reported by Rundle[5]. Crystals having an identical external form were always obtained in all of our experiments. A marked dichroism was observed under the microscope. The crystal is yellow with the electric vector along the *c*-axis and brownish orange with the electric vector normal to the *c*-axis. The dichroism measurement was carried out with polarized light having its electric vector along and normal to the *c*-axis. The *c*- and *a*-absorption represent the absorption with the electric vector along and normal to the *c*-axis, respectively.

Measurement.—Quantitative dichroism measurement was done by Tsuchida-Kobayashi's microscopic method using polarized light[6]. The symbols used here are the same as those in our former papers of this series.

Results and Discussion

The result of the dichroism measurement is shown in Fig. 1 and Table I. According to the crystal structure analysis of $[Au(dgH)_2][AuCl_2]$ reported by Rundle, the planar cations of $[Au(dgH)_2]^+$ and linear anions of $[AuCl_2]^-$ are arranged alternately in the crystal of the present compound such that the planes and the lines of the complex ions are parallel to each other and perpendicular to the *c*-axis, that is, the needle axis. The *c*-absorption is seen to correspond with the $\perp$-absorption, the absorption with the electric vector normal to the direction in which lie the linkages of the ligands with the metallic ion. The *a*-absorption is found to represent the main features of the $\parallel$-absorption of the complex ion; that is, the absorption with the electric vector along the linkage.

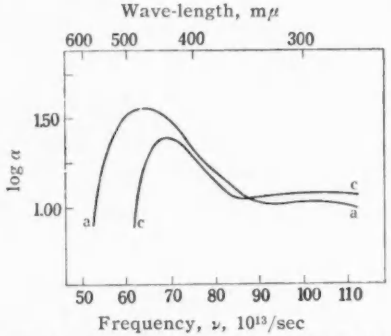

Fig. 1. Absorption spectra of $[Au(dgH)_2][AuCl_2]$ in the crystalline state.

The present measurement of dichroism shows that *for the absorption band at the longer wave-length, the $\parallel$-absorption is about four and a half times as intense as the $\perp$-absorption.*

From the short Au(I)-Au(III) distance of 3.23 Å, Rundle[5] suggested the existence of weak linkage between the Au(I) and Au(III) ions in the present compound. The weak metal-to-metal linkage as proposed for the present compound would be somewhat similar to the metal-to-metal linkage for Magnus's green salt, bis-(dimethylglyoximato)-nickel(II), and so forth. Our former researches[3] indicate that for the absorption band at the longer wave-length of those compounds involving the metal-to-metal interaction, absorption is stronger in the direction normal to the complex plane; i. e. in the direction in which the metal-to-metal linkage is expected to exist than along the plane of the complex. This is not the case with the present compound. Thus the present dichroism measurement seems to indicate that *the metal-to-metal interaction may not exist between the Au(I) and the Au(III) ions in the present compound.*

TABLE I

ABSORPTION MAXIMA OF $[Au(dgH)_2][AuCl_2]$

	ν, 10^{13}/sec.	log α	half width, 10^{13}/sec.
a-abs.	64	1.55	18
c-abs.	69	1.37	6.5

Assuming the absence of the metal-to-metal interaction between the Au(I) and the Au(III) ions, the absorption band at about $60\text{–}70\times10^{13}$/sec. may be due to the planar $[Au(dgH)_2]^+$ ions under slight perturbation from the surrounding ions, since the $[AuCl_2]^-$ ion is not expected to show absorption in this wave-length region. For planar metallic complexes involving no metal-to-metal interaction, previous studies[7] show that with the absorption band at the longer wave-length, electric vector is more strongly absorbed along the plane of the complex than along the direction normal to the complex plane.

5) R. E. Rundle, *J. Am. Chem. Soc.*, **76**, 3101 (1954).
6) R. Tsuchida and M. Kobayashi, "The Colour and the Structure of Metallic Compounds", Zoshindo, Osaka, 1944, p. 180. See also the previous papers of this series.
7) S. Yamada and R. Tsuchida, This Bulletin, **26**, 489 (1953); ibid., **29**, 289 (1956); ibid., **29**, 694 (1956); *Ann. Rep. Scient. Works, Fac. Sci. Osaka Univ.*, **4**, 79 (1956), etc.

A similar relationship may reasonably be expected for the dichroism of the planar $[Au(dgH)_2]^+$ ion. The relationship obtained with $[Au(dgH)_2][AuCl_2]$ is seen to agree with that for typical planar complexes without the metal-to-metal interaction. This fact is also compatible with the conclusion that the metal-to-metal interaction is absent in the present compound.

It is to be noted that the metal-to-metal interaction is absent in the crystal of $[Au(dgH)_2][AuCl_2]$, where the Au(I)-Au(III) distance is comparatively short and almost equal to the Ni-Ni distance in the crystal of $Ni(dgH)_2$, in which the metal-to-metal interaction was concluded to exist.

This difference between $[Au(dgH)_2][AuCl_2]$ and $Ni(dgH)_2$ will be discussed in the following part of the present paper. The ionic radius of Ni(II) in the molecule of $Ni(dgH)_2$ is expected to be larger than that of Au(III) in the ion of $[Au(dgH)_2]^+$, judging from difference in the electronic charge between them. This might be one of the reasons for the absence of the metal-to-metal interaction.

In addition to this, however, there may be another reason for this phenomenon, which we are inclined to regard as more important. The central nickel ion in the $[Ni(dgH)_2]$ molecule is assumed to have non-bonding *d*-electrons spreading in the regions above and below the metallic ion in the direction normal to the plane of the complex. These non-bonding *d*-electrons may be available for some additional bonding, since a very great amount of energy may not be required to promote these electrons for the additional bonding. It was assumed in the former papers[3,8] that the nickel ions lying at an equal distance along the *c*-axis form octahedral sp^3d^2 hybridization orbitals, overlapping of the orbitals being responsible for the metal-to-metal interaction. For the overlapping of the orbitals resulting in the metal-to-metal linkage may be used the non-bonding 3*d*-electrons which originally spread in the direction perpendicular to the plane. This seems to be consistent with the idea proposed by Rundle and Sturdivant[9], who pointed out that the existence of a low-lying orbital was necessary for the metal-to-metal linkage. The existence of electrons which can be promoted for the linkage with a comparatively small amount of energy may also be important.

As for $[Au(dgH)_2][AuCl_2]$, the $[Au(dgH)_2]^+$ ion involves similar situation to that of $[Ni(dgH)_2]$, the above condition for the metal-to-metal linkage being satisfied. On the contrary, the Au(I) ion in the $[AuCl_2]^-$ ion forms linear *sp* hydridization orbitals in the outside of the $5d^{10}$ closed shell. For the metal-to-metal linkage, the Au(I) must form square hybridization orbitals and at the same time electrons in the $5d^{10}$ closed shell must be available. We prefer to suppose that the considerably great stability of the $5d^{10}$ closed shell might make the above conditions difficult to be fulfilled. Thus the conclusion that the Au(I)-Au(III) interaction is absent in the present compound may be understood on the basis of these considerations.

In advancing the hypothetical metal-to-metal linkage in the present compound, Rundle[5] assumed $5d6s6p^2$ hybridization orbitals of the Au(I) ion which would result in overlapping with some of the hybridization orbitals of the Au(III) ion. A comparatively great amount of energy may be required for the $5d6s6p^2$ hybridization because of the possibly great stability of the $5d^{10}$ closed shell. In our view on the basis of the present observation, the hybridization of $5d6s6p^2$ can hardly be assumed to occur at such a distance as observed for the crystal of the present compound.

Summary

Dichroism of $[Au(dgH)_2][AuCl_2]$ has been determined in the visible and the ultraviolet region by the microscopic method. For the absorption band at about $60–70\times10^{13}$/sec., absorption has been found to be about four and a half times as strong along the complex plane as along the direction normal to the plane.

From discussion on the result of the dichroism measurement, it has been concluded that the metal-to-metal interaction does not exist between the Au(I) and Au(III) ions in this crystal.

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8) L. E. Godycki and R. E. Rundle, *Acta Cryst.*, **6**, 487 (1953).
9) R. E. Rundle and J. H. Sturdivant, *J. Am. Chem. Soc.*, **69**, 1561 (1947).

*The Formation of Cellulose IV in the Viscose Spinning**

By Yoshizo TSUDA and Sadataka MUKOYAMA

(Received April 17, 1957)

It has been generally acknowledged that the crystal form of the cellulose in viscose rayon may be designated as cellulose II. However, as pointed out by Ingersol[1] and Howsmon[2] a small amount of cellulose IV may also be formed during the spinning of viscose rayon under special conditions, although those conditions have not been elucidated. Thus the mechanism of the cellulose IV formation in the spinning of viscose rayon is still unknown.

One will be able to find the presence of cellulose IV in the X-ray diagram of tire cord rayon, while none can be found in that of textile rayon. This fact suggests that the formation of cellulose IV may be facilitated by the spinning process characteristic of the spinning of tire cord rayon. It has been the purpose of this work to investigate the mechanism of the formation of cellulose IV in the spinning of tire cord rayon.

It has been suggested[2] that cellulcse IV may be formed by the direct decomposition of cellulose xanthates. If this is true, the zinc cellulose xanthate formed temporarily in the viscose spinning process may play an important role in the formation of cellulose IV. According to this suggestion the present work was started with the investigation of the regeneration of zinc cellulose xanthate model filament and it was shown that high temperature of regeneration is the necessary condition to form cellulose IV in this case. The mechanism of the formation of the cellulose IV in the viscose spinning process was investigated with the experimental rayon spun by the stretch spinning method on the basis of the conclusion obtained above.

The Crystal Structure of Cellulose IV

It has been confirmed by a number of investigations[3] that cellulose I is stable at higher temperatures and can be formed by transforming cellulose IV at higher temperatures by various methods. The unit cell of cellulose is described as rhombic with the dimension a=8.11 Å, b=10.3 Å, c=7.9 Å and the angle β approximately 90°. The principal X-ray evidence for the existence of cellulose IV lies in the fact that only one equatorial interference occurs in place of the 101, 101 doublet of cellulose I. As pointed out by Howsmon[2], cellulose IV may be regarded as a disordered form of cellulose I.

Experimental

(a) **Cellulose Samples.**—Ammonium cellulose xanthate model filament was prepared according to the procedure employed by Hermans[4]. Zinc cellulose xanthate model filament was obtained by immersing ammonium cellulose xanthate model filament in a cold saturated zinc sulfate solution for half an hour,, stretching to twice its original length and then regenerating. Dilute sulfuric acid solutions at various temperatures (40°C, 60°C, 80°C, 100°C) as well as boiling water and glycerine at 150°C were employed for the regeneration.

Experimental rayon was spun in Müller-type baths of various compositions followed by a second bath either at a higher temperature (95°C) or at a lower temperature (60°C). In the following table the compositions of the Müller-type baths employed and the corresponding temperature of the second bath are shown.

TABLE I
SPINNING CONDITIONS OF EXPERIMENTAL RAYON

No. of Sample	Composition of Müller-type bath wt. %			Temp. of second bath °C
	H_2SO_4	Na_2SO_4	$ZnSO_4$	
I	8	20	1	60
II	8	20	1	95
III	8	20	2	60
IV	8	20	2	95
V	8	20	3	60
VI	8	20	3	95
VII	8	20	4	60
VIII	8	20	4	95

* The main part of this paper was presented at the 10th Annual Meeting of the Chemical Society of Japan. April 1957.

1) H. G. Ingersol, *J. Applied Phys.*, **11**, 924 (1946).

2) J. A. Howsmon, W. A. Sisson, "Cellulose and Cellulose Derivatives" (E. Ott) Part 1. pp. 241-244 (1954).

3) K. Hutino and I. Sakurada, *Naturwissenschaften* 577 (1940); T. Kubo, *Z. phisik. Chem.*, **A 49**, 297 (1940); K. Hess and H. Kiessig, *Z. phisik. Chem.*, **B 49**, 235 (1941).

4) P. H. Hermans, "Physics and Chemistry of Cellulose Fibres", p. 439 (1949).

The temperature of the first bath (Müller-type bath) was kept at 50°C, the spinning speed at 25 m. and the first bath travel at 30 cm.

(b) X-Ray Diffraction.—The Norelco diffractometer of the North American Philips Company, Inc., with auxiliary equipments, consisting of a recording potentiometer and a scaler counting unit, was used in these studies. Nickel-filtered radiation from a copper target operated at 35 kV and 15 mA tube current was used.

In order to obtain pulverous specimens, the cellulose samples were methanolized for a day with one normal hydrochloric acid in absolute methyl alcohol at 40°C. This treatment gave a ninety-five per cent. yield of cellulose.

No change in the crystal form through the methanolysis was confirmed by comparing the X-ray diffraction intensity curve of the methanolized sample with the X-ray diffraction diagram of the fibrous sample.

Results and Discussion

The X-ray diffraction intensity curves of textile rayon and tire cord rayon are shown in Fig. 1. No peak of cellulose IV appears in the diffraction intensity curve of textile rayon. In contrast, a remarkable peak of cellulose IV is observed in the diffraction intensity curve of tire cord rayon. The difference of this nature will be found between any textile and tire cord rayons.

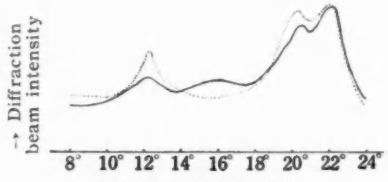

Fig. 1. X-ray diffraction intensity curves of tire cord rayon (full line) and textile rayon (dotted line).

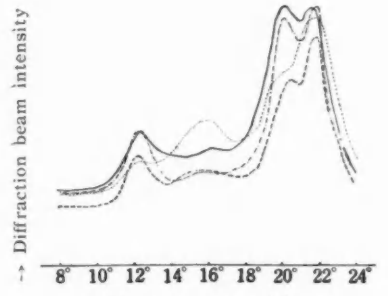

Fig. 2. X-ray diffraction intensity curves of model filaments regenerated at various temperatures: 40°C (full line), 60°C (chain line), 80°C (broken line) and 100°C (dotted line).

The X-ray diffraction intensity curves of the model filaments regenerated with dilute sulfuric acid solutions at various temperatures are shown in Fig. 2. It is seen that the relative intensity of the peak of cellulose IV increases slightly with the increase in the temperature of regeneration from 40°C to 80°C, while a marked increase of cellulose IV is observed with the sample regenerated at 100°C. It should be mentioned that the critical temperature which determines the formation of cellulose IV lies between 80°C and 100°C.

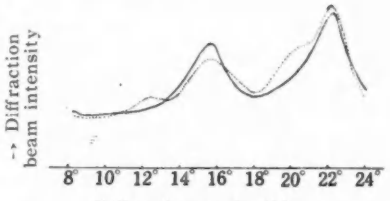

Fig. 3. X-ray diffraction intensity curves of model filaments regenerated with boiling water (dotted line) and regenerated with glycerine at 150°C (full line).

The X-ray diffraction intensity curves of the model filaments regenerated with boiling water and with glycerine at 150°C are shown in Fig. 3. The curve for the sample regenerated with boiling water is identical with that for the sample regenerated with dilute sulfuric acid at 100°C. This fact indicates that the formation of cellulose IV depends solely on the temperature of regeneration.

The curve for the sample regenerated with glycerine at 150°C shows that the crystal form of the sample consists of cellulose IV alone. It may be concluded that the degree of formation of cellulose IV by the decomposition of zinc cellulose xanthate is determined by the temperature and that the regeneration temperature above a critical temperature which is between 80°C and 100°C, is required for the formation of cellulose IV.

The X-ray diffraction intensity curves of the experimental rayon are shown in Fig. 4. It can be seen from those curves that the formation of cellulose IV depends on the temperature of the second bath. Namely the samples which passed through the second bath at 95°C contain the crystal

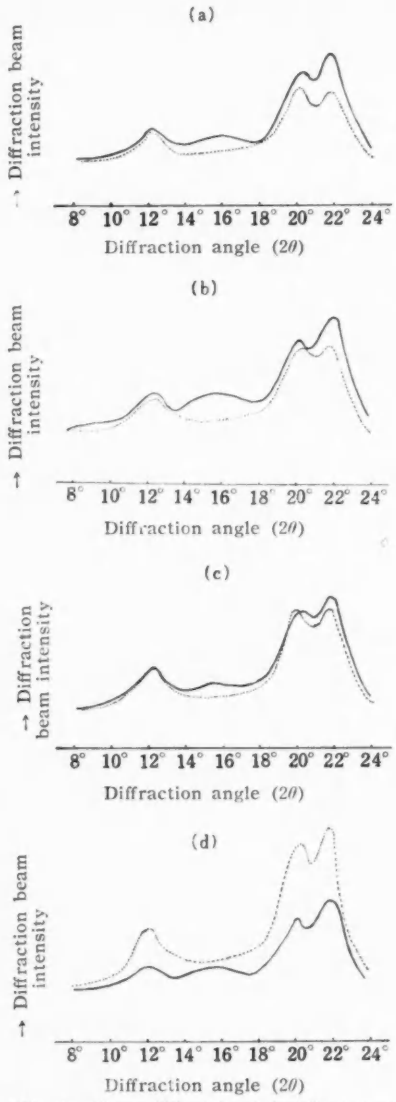
(a)
Diffraction beam intensity
8° 10° 12° 14° 16° 18° 20° 22° 24°
Diffraction angle (2θ)
(b)
Diffraction beam intensity
8° 10° 12° 14° 16° 18° 20° 22° 24°
Diffraction angle (2θ)
(c)
Diffraction beam intensity
8° 10° 12° 14° 16° 18° 20° 22° 24°
Diffraction angle (2θ)
(d)
Diffraction beam intensity
8° 10° 12° 14° 16° 18° 20° 22° 24°
Diffraction angle (2θ)

Fig. 4. X-ray diffraction intensity curves of experimental rayon: (a) I, II, (b) III, IV; (c) V, VI; (d) VII, VIII; here I, III, V and VII are drawn in dotted lines and the rests in full lines.

form of cellulose IV, while the samples which passed through the second bath at 60°C contain no crystal form of cellulose IV. In addition, the relative intensity of the peak of cellulose IV as compared of cellulose II increases with that with the increase in the concentration of zinc sulfate in the first bath. These facts will be elucidated as follows. The zinc cellulose xanthate which is formed temporarily in the first bath is brought into the second bath maintained at a higher temperature where it is regenerated. Thus the crystal form of the cellulose IV is included in the sample which passed through the second bath at a sufficiently high temperature. The formation of zinc cellulose xanthate depends on the concentration of zinc sulfate in the first bath. Consequently, the higher the concentration of zinc sulfate, the greater the content of cellulose IV in the regenerated sample.

It may be concluded from the results that the partical formation of cellulose IV in tire cord rayon is due to the higher temperature of the second bath and the higher content of zinc sulfate in the first bath.

Summary

The formation of cellulose IV in the viscose spinning process was investigated by the X-ray diffraction measurements. Zinc cellulose xanthate model filaments were regenerated at various temperatures and their X-ray diffraction diagrams were examined. It was observed that the formation of cellulose IV is remarkable when zinc cellulose xanthate is regenerated at the temperature above a critical temperature which lies between 80°C and 100°C. The mechanism of partial formation of cellulose IV in the viscose spinning process was elucidated as follows: when zinc cellulose xanthate formed in the first bath is regenerated at a sufficiently high temperature in the second bath, the formation of cellulose IV consequently results. The main factors involved for the formation of cellulose IV are higher temperature of the second bath and higher concentration of zinc sulfate in the first bath.

The authors are grateful to Dr. K. Hoshino for premission of this publication. The authors sincer thanks are also due to Professor K. Maeda of Tokyo Institute of Technology and Mr. T. Yurugi of the Textile Research Institute for their kind discussions.

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Studies on Carbon-Chlorine Bonds in Some Aromatic Compounds by Their Pure Quadrupole Spectra

By Hisao NEGITA, Sumio SATOU, Teijiro YONEZAWA and Kenichi FUKUI

(Received April 25, 1957)

Pure quadrupole spectra of many chlorine compounds have been reported so far and their resonance absorption frequencies or nuclear quadrupole coupling constants have been interpreted from various points of view[1]. Some years ago, Meal[2] found a nearly linear relationship between the frequencies and Hammett's σ of various substituents in monosubstituted chlorobenzene. Later, the same result as this has been recognized also in bromobenzene, iodobenzene[3], and dichlorobenzene[4].

In this paper we studied other effects on the frequencies, that is, the frequency shifts which resulted from replacement of one or more carbon atoms in the benzene ring by the corresponding numbers of nitrogen atoms. The compounds used for comparison were chlorobenzene, 2-chloropyridine, 1,3,5-trichlorobenzene, and cyanuric chloride. As the frequencies due to ^{35}Cl of the first three compounds were already known[5~7], we measured only those of the last, which were closely spaced and higher than those of trichlorobenzene. This result is evidently contrary to that of chloropyridine, where its frequency is lower than that of chlorobenzene. However, these opposing phenomena due to nitrogen atoms may be correlated with each other by the theoretical consideration, for the decrease of π-electron density at chlorine atoms, which accompanies that of σ-electron necessarily, is much more remarkable in the former than in the latter, compared to the increase of bond order of carbon-chlorine bonds.

Experimental

Sample.—To obtain cyanuric chloride $(CNCl)_3$, cyanuric acid $(CNOH)_3$ was prepared from urea and anhydrous zinc chloride, and then it was chlorinated by phosphorus pentachloride in phosphorus oxychloride as the solvent[8,9]. The product was purified by recrystallization from chloroform and then by sublimation in a long test tube. The sample thus obtained melted around 146°C, which was in good agreement with the value already known.

Apparatus.—The resonance absorption frequencies due to ^{35}Cl were measured by the frequency modulated super-regenerative spectrometer described in the previous paper[4,10] and was ascertained by those due to ^{37}Cl, which should be found at the lower frequency region[11]. To obtain the data at lower temperature, solid carbon dioxide and toluene were used as the freezing mixture, and the temperature was measured with copper-constantan and a potentiometer.

The experimental error in frequency would be introduced from reading off both frequency and temperature of the sample, and amounts to several kilocycles/sec., which might not be so serious in the following considerations.

Results and Discussions

The experimental results are shown in Table I. From this table, it is seen that two resonance absorption lines are so closely spaced, especially at a lower temperature, that they are observed without any interferences from side bands.

TABLE I

NUCLEAR QUADRUPOLE RESONANCE FREQUENCIES (ν) DUE TO ^{35}Cl OF CYANURIC CHLORIDE

Temp.	195°K	285°K
ν (Mc./sec.)	36.533—Weak 36.537—Strong	36.323—Strong 36.338—Weak

At room temperature, 285°K, the lower line was much stronger than the other, which would tell the fact that the chlorine atoms corresponding to the former are

1) H. Negita, *Chem. and Chem. Ind., Japan*, **8**, 415 (1955).
2) H. C. Meal, *J. Am. Chem. Soc.*, **74**, 6074 (1952).
3) J. Hatton and B. V. Rollin, *Trans. Faraday Soc.*, **50**, 398 (1954).
4) H. Negita, H. Yamamura and H. Shiba, This Bulletin, **28**, 271 (1955).
5) R. Livingston, *Phys. Rev.*, **82**, 289 (1951).
6) S. Segel, R. G. Barnes and P. J. Bray, *J. Chem. Phys.*, **25**, 1286 (1956).
7) P. J. Bray, R. G. Barnes and R. Bersohn, *J. Chem. Phys.*, **25**, 813 (1956).
8) Beilsteins Handbuch der Organischen Chemie, 4 Aufl., Julius Springer, Berlin, (1937), XXVI, p. 35.
9) Z. Yoshida and R. Oda, *J. Chem. Soc., Japan, Ind. Chem. Sec.*, **56**, 92 (1953).
10) H. Negita and S. Satou, This Bulletin, **29**, 426 (1956).
11) R. Livingston, *J. Phys. Chem.*, **57**, 496 (1953).

are more abundant than the others, the ratio presumanbly being 2 : 1. However, the situation is reversed at 185°K. Thus, it may be concluded that the two lines are different from each other in the temperature gradient and they seem to overlap at some intermediate temperature, or else there are some transition points. In spite of this extraordinary behavior, our present interest is in the following fact.

The average value of the frequencies of cyanuric chloride is higher than that of trichlorobenzene, taking account of the temperature effect. To see this more precisely, the two average values in Table I were extrapolated to 77°K and about 36.725 Mc./sec. was obtained as the value at that temperature. It is obviously higher than that of trichlorobenzene[7] and this fact is contrary to the case of 2-chloropyridine[6] and chlorobenzene[5].

For comparison, the frequencies of these compounds are tabulated in Table II. At first sight, the replaced nitrogen atom in the ring will be thought to attract more electrons from the chlorine atom, with the result that the electron density around the latter is decreased, which in turn increases the frequency. However, this is not the case in the first row of Table II. Accordingly, the decrease in the frequency may be attributed to the increase in double bond character induced simultaneously, as pointed out by Segel et al.[6]

TABLE II

NUCLEAR QUADRUPOLE RESONANCE FREQUENCIES (ν) DUE TO ^{35}Cl OF SOME AROMATIC COMPOUNDS AT 77°K

Compd.	ν (Mc./sec.)	Compd.	ν (Mc./sec.)
Chlorobenzene	34.6216	2-Chloropyridine	34.194
1,3,5-Trichlorobenzene	35.851 (Average)	Cyanuric chloride	36.725 (Average)

On the contrary, the increase in the frequency in the second row is supposed to result from the decrease in electron density, compensating the increase in the double bond character of carbon-chlorine bond. As a rule, circumstances around a molecule in the solid are more complex than in the vapor, owing to the neighboring molecules. However, such compounds as listed in the same row of Table II may be compared with each other by their isolated molecules, since they form molecular crystals alike in size and shape. Thus it will be worth while to examine the data of pure quadrupole spectra with reference to electronic structures computed from molecular orbital theory.

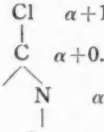
Now the ionic character and double bond character of carbon-chlorine bond may be considered in terms of π-electron density (q) at chlorine atom and mobile bond order (p) of the bond, respectively[12]. These two quantities are calculated in Table III for the compounds concerned.

TABLE III

π-ELECTRON DENSITIES (q) AT CHLORINE ATOMS AND MOBILE BOND ORDERS (p) OF CARBON CHLORINE BONDS

Compd.	q & p	Compd.	q & p
Chlorobenzene	$q=1.9225$ $p=0.2728$	2-Chloropyridine	$q=1.9084$ $p=0.3391$
1,3,5-Trichlorobenzene	$q=1.9209$ $p=0.2744$	Cyanuric chloride	$q=1.8650$ $p=0.4167$

Parameters adopted in calculation are as follows. Coulomb integrals are such that



and resonance integral of C—Cl is 0.8 β, where α and β are the Coulomb and the resonance integral in benzene, respectively.

As seen in Table III, the decrease in the π-electron density at chlorine atom in the second row amounts to about four times as much as that of the first row, whereas the increase in carbon-chlorine bond order in the former is only twice as large as that of the latter. From these figures, the resonance frequency may decrease or increase as illustrated in Fig. 1,

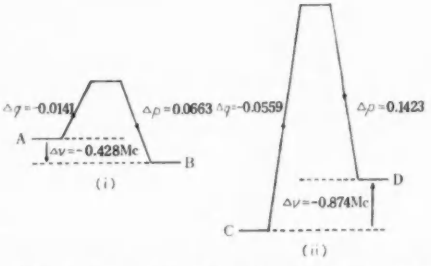


Fig. 1. Correlation of molecular constants and nuclear quadrupole resonance frequencies due to ^{35}Cl.
(i) A···chlorobenzene,
B···2-chloropyridine.
(ii) C···1,3,5-trichlorobenzene,
D···cyanuric chloride.

12) C. A. Coulson and H. C. Longuet-Higgins, *Proc. Roy. Soc.* (*London*), **A191**, 39 (1937).

where A, B, C and D represent chlorobenzene, 2-chloropyridine, 1,3,5-trichlorobenzene, and cyanuric chloride, respectively.

It may be noted that the decrease in the π-electron density at a chlorine atom accompanies the decrease in the σ-electron and results in an increase in the frequency. As seen in Fig. 1, this increase is assumed for convenience to be proportional to the decrease in the former, for there is no straightforward way to compute the latter, but the frequency shifts are not so large in our cases. Thus, from the simplified schemes, it follows that $\Delta\nu/\Delta q \simeq -69$ Mc./sec. and $\Delta\nu/\Delta p \simeq -21$ Mc./sec..

The absolute value of the former is relatively large, though it includes the effect of the σ-electron. However, it is very difficult to justify the value. Thus it will be fair only to say that there is considerable transfer of the σ-electron accompanied with the π-electron. On the other hand, the latter value may be accepted easily, for it is near to the value -27.4 Mc./sec., which is computed as follows. From the experimental value of the coupling constant of free chlorine atom[13] and the number of unbalanced p-electrons, the frequency is assumed to be 54.8 Mc./sec. for pure $-\mathrm{Cl}$ state and $54.8\times\frac{1}{2}=27.4$ Mc./sec. for $=\mathrm{Cl}^+$, while the bond order is 0 for the former and 1 for the latter, so that $\Delta\nu/\Delta p = -27.4$ Mc./sec. when $\Delta p=1$. It will be interesting to see whether the above relations might hold also in other cases, or not.

The authors wish to express their sincere thanks to Professor T. Ichikawa and Dr. H. Yamamura for their encouragement throughout this research, and also to Messers F. Okada and Z. Hirano for their able assistance to this work.

Faculty of Science, Hiroshima University, Hiroshima Faculty, of Education Kagawa University, Takamatsu, and Faculty of Engineering, Kyoto University, Kyoto

13) V. Jaccarino and J. G. King, *Phys. Rev.*, **83**, 471 (1951).

An Account on the Infrared Absorption of Guanidiniums

By Toshio GOTO, Koji NAKANISHI and Mamoru OHASHI

(Received April 25, 1957)

The infrared spectra of several guanidine hydrochlorides have been examined in connection with the structural elucidation of roseonine[1] and roseothricin[2]. Except for a few scattered data[3] it seems that as yet no systematic study has been undertaken on the infrared absorption of guanidines, and accordingly, though the studies are far from comprehensive, results of diagnostic value are reported herein. The spectra are summarised in Fig. 1. The apparent general tendency is that with unsubstituted and symmetrically trisubstituted guanidine hydrochlorides (Nos. 1, 9 and 10) there is only a single strong absorption around 1650 cm^{-1}, whilst with mono- and disubstituted guanidine hydrochlorides (Nos. 2—8) there are two conspicuous bands in the region 1700—1580 cm^{-1}. Furthermore the separation between the two bands is generally wider with disubstituted guanidiniums (ca. 90 cm^{-1}) than with monosubstituted guanidiniums (ca. 20 cm^{-1}). For the sake of convenience we would like to refer to these bands as the guanidinium bands, and in cases when splitting is observed as the guanidinium I (higher frequency) and II (lower frequency) bands. The guanidinium band(s) apparently corresponds to the band around 1660 cm^{-1} of free guanidines which is assigned to the C=N stretching[4]. With guanidine hydrochlorides it would be due to an antisymmetric stretching of the carbon nitrogen bonds in the guanidinium

1) K. Nakanishi and M. Ohashi, following paper.
2) T. Goto, Y. Hirata, S. Hosoya and N. Komatsu, following paper.
3) H. M. Randall, R. G. Fowler, N. Fuson, J. R. Dangl, "Infrared Determ. of Org. Structures", D. van Nostrand (1949).

4) E. Lieber, D. R. Levering and L. Patterson, *Anal. Chem.*, **23**, 1594 (1951).

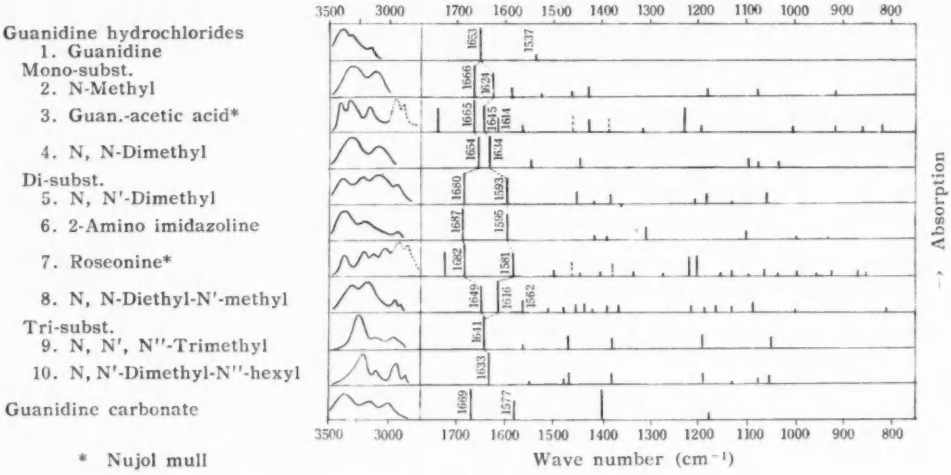
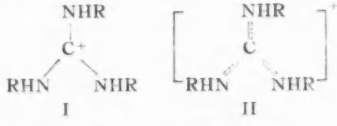


Fig. 1

group, probably coupled to some extent with NH_2^- bending vibrations[5]. A single antisymmetric carbon nitrogen stretching vibration would be expected to be infrared active with unsubstituted and sym. trisubstituted guanidiniums, whilst two such vibrations would be expected with mono- and disubstituted guanidiniums; these expectations are thus in agreement with experimental observations. Since the symmetric carbon nitrogen stretching vibration of guanidiniums reveals itself as a strong absorption around 1000 cm^{-1} in the Raman spectra[6], some of the weak infrared bands around 1000 cm^{-1} could possibly be due to this sort of vibration; however they are of no diagnostic value. The absorption of guanidine carbonate (No. 11) which show bands at 1669 and 1577 cm^{-1} as compared to the single band at 1653 cm^{-1} of guanidine hydrochloride is anomalous. Since the spectra were all recorded in the solid state this is probably due to the influence of the negative ion on the crystal structure, and accordingly the results mentioned in the present paper are valid only for the hydrochlorides.

The relationship between an amino group and a positively charged ammonium group is probably of a continuous nature with no qualitative difference between them. Taking into account the fact that typical ammonium absorptions (N$^+$H stretching) occur below ca. 3200 cm^{-1}[5], whereas typical amine absorptions (N–H stretching) occur above 3200 cm^{-1}, the following generalisations regarding charge distribution within the guanidinium group may be inferred from the absorption of the guanidiniums in the 3000 cm^{-1} region. Namely, the position of the absorptions in this region (Fig. 1.) corresponds to those of free amines rather than to amine salts, and this suggests that the positive charge resides mainly on the central carbon atom (I) rather than being distributed over the entire guanidinium group (II). This is further supported by the



fact that the absorptions of sym-trisubstituted guanidine hydrochlorides (Nos. 9, 10) which, according to structure I, would mainly possess only —NHR groups, reveal themselves as a more or less single major band, whereas those of other guanidiniums (which sould possess —NH_2 groups as well) reveal themselves as a broad band with two or more maxima, a behavior similar to that of free secondary and primary amines, respectively.

Experimental

The guanidines were prepared according to the following methods.

5) A. Yamaguchi, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **78**, 140 (1957). The authors are greatly indebted to Ass. Profs. T. Shimanouchi (Tokyo Univ.) and M. Kimura (Nagoya Univ.) for valuable suggestions.

6) J. T. Edsall, *J. Phys. Chem.*, **41**, 133 (1937).

7) K. Nakanishi, T. Goto and M. Ohashi, This Bulletin, **30**, 403 (1957).

N-Methyl guanidine picrate, m. p. 196—198° [8].

N, N-Dimethyl guanidine picrate, m. p. 216—218° [8].

N' N'-Dimethyl guanidine picrate, m. p. 174—175° [9].

2-Aminoimidazoline picrate, m. p. 217°.

N, N-Diethyl-N'-methyl guanidine was prepared by the condensation of methyl isothiocyanate and dimethylamine, followed by treatment with ammonia and mercuric oxide in absolute ethanol solution. This is a new compound. Picrate, yellow plates, m. p. 128—129°.

Anal. Found: C, 40.60, 40.55, H, 4.88, 5.12, N, 23.19, 23.38%. Calcd. for $C_{12}H_{13}O_7N_6$: C, 40.22, H, 5.06. N, 23.46.

N, N', N''-Trimethyl guanidine picrate, m. p. 210—212° [10].

N, N'-Methyl-N''-*n*-hexyl guanidine was prepared by the treatment of N-methyl-N'-*n*-hexyl thiourea with methylamine and mercuric oxide in absolute ethanol. This is also a new compound but the picrate could not be obtained crystalline, and the crude hydrochloride was submitted directly to infrared measurements.

The picrates were treated with dilute hydrochloric acid, the liberated picric acid was extracted with ether, and the aqueous layer was evaporated to dryness in vacuo to give the respective hydrochlorides.

Infrared absorption measurements. The spectra were recorded on a Hilger H 800 double beam instrument equipped with a sodium chloride prism. Potassium bromide disks were used, and the die and the handpress were those supplied by Hilger and Watts, Co. Analytical grade potassium bromide was ground to pass a 200 mesh sieve and dried at 150°C for 24 hours; the samples (ca. 0.5 mg.) were ground evenly with 300 mg. of this potassium bromide for 5 minutes.

The authors are indebted to Professor Y. Hirata for his interest in this research.

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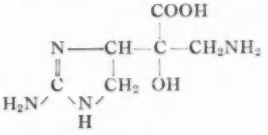
8) E. A. Werner and J. Bell, *J. Chem. Soc.*, **1922**, 1790; E. Philippi and K. Morsch, *Ber.*, **60**, 2120. (1927).
9) T. L. Davis and E. N. Rosenquist, *J. Am. Chem. Soc.*, **59**, 2112 (1937).
10) T. L. Davis and R. C. Elderfield, *J. Am. Chem. Soc.*, **54**, 1499 (1932).

*Confirmation of Structure of Roseonine**

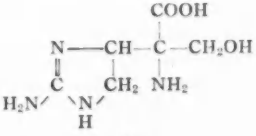
By Koji NAKANISHI and Mamoru OHASHI

(Received April 25, 1957)

Roseonine[1] is one of the two β-amino acids obtained as the hydrolysis products of roseothricin[2,3], and is identical with streptolidine from streptolin[4] and geamine from geomycin[5]; it has also recently been obtained from racemomycin B[6]. On account of the poor crystallizing properties of the reaction products from roseonine, and especially because the starting material was so scarce, the reasoning that led to the previously proposed structure I[1,7] was somewhat indirect. The validity of structure I and the possibilities of three



(I)



(II)

* Reported at 9th Annual Meeting, *Chem. Soc. Japan*, *Kyoto*, April, 1956.

1) K. Nakanishi, T. Ito, M. Ohashi, I. Morimoto and Y. Hirata, This Bulletin, **27**, 539 (1954).
K. Nakanishi, T. Ito and Y. Hirata, *J. Am. Chem. Soc.*, **76**, 2845 (1954).
K. Nakanishi, *J. of Antibiotics*, Ser. B., VII-7, 228 (1954).

2) S. Hosoya, M. Soeda, N. Komatsu, S. Imamura, M. Iwasaki, Y. Sonoda and K. Okada, *Jap. J. Exp. Med.*, **20**, 121 (1949); *J. of Antibiotics*, Ser. B, III-4, 217 (1950).

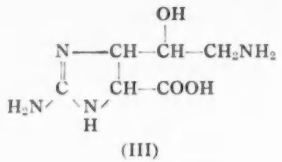
3) T. Goto, Y. Hirata, S. Hosoya and N. Komatsu, following paper.

4) H. E. Carter, W. R. Hearn and W. R. Taylor, "Abstracts of Papers", 120th Meeting, Am. Chem. Soc., New York, N. Y., September, 1951, p. 31.
E. E. Smissmann, R. W. Sharpe and E. E. van Tamelen, "Abstracts of Papers", 121st Meeting, Am. Chem. Soc., Milwaukee, Wisc., April 1952, p. 80.
Identity of roseonine with streptolidine confirmed by direct comparison of infrared spectra (personal communication from Dr. van Tamelen).

5) H. Brockamnn and H. Musso, *Ber.*, **88** 648 (1955).

6) H. Taniyama and S. Takemura, "Abstracts of Papers", 10th Annual Meeting, Pharm. Soc. Japan, Tokyo, April, 1957, p. 88.

7) For simplicity the structures I-IV are represented in the classical fashion and not in the zwitterionic form in which distribution of the positive charge within the guanidinium moiety should have to be considered. However, see structure I of preceding paper.



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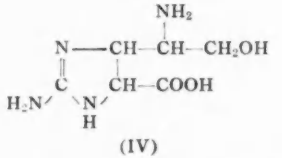
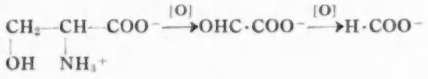


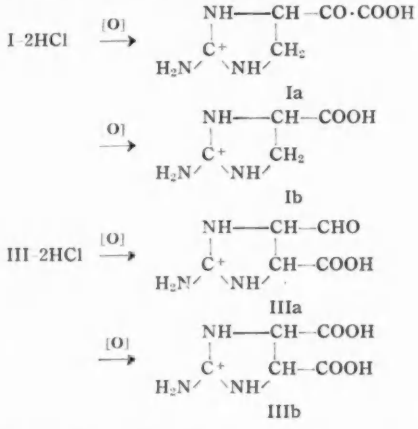
(IV)

other structures, II–IV, have accordingly been re-investigated. In the present paper we wish to furnish additional evidence pointing to structure I, and thus to clarify a few ambiguities[8]. Firstly, potentiometric titration and consideration of the hydrogen ion-binding curve[9] excluded structures III and IV, and secondly, evidence from infrared spectra pointed to the correctness of structure I.

Free D,L-serine was chosen as the model for the first method, and this was subjected to a two-mole periodate oxidation. Potentiometric titration of the aqueous reaction solution gave a pK'_a value of 3.8, and the hydrogen ion-binding curve occurred ***below*** the zero reference line (i.e., the inflection corresponded to a hydrogen ion-binding group). The dissociation constant corresponded to that of formic acid, and thus the two oxidative steps may be correctly formulated by the following scheme:



Oxidation of roseonine dihydrochloride with one mole periodate yielded one mole each of formaldehyde and ammonia[1] and an unisolated product with a pK'_a of 2.1[10]. Oxidation with a second mole of periodate afforded a product with a pK'_a value of 3.3. Since neither formaldehyde nor 2-amino-2-imidazoline hydrochloride was attacked by periodate to any measurable extent under similar conditions, it is apparent that the inflection at 3.3 is neither due to the transformation of formaldehyde to formic acid, nor to an oxidative decomposition of the 2-imidazoline nucleus. Hence the oxidative steps may be represented as follows for structures I (or II)[11] and III (or IV)[11], respectively:



The oxidation product could not be isolated from the reaction solution owing to poor crystallizing properties and the minute amount of starting material available. Otherwise a simple analysis would have sufficed to differentiate between structures Ib and IIIb. Though no clear differentiation between structures Ia and IIIa, or between Ib and IIIb is possible from the two

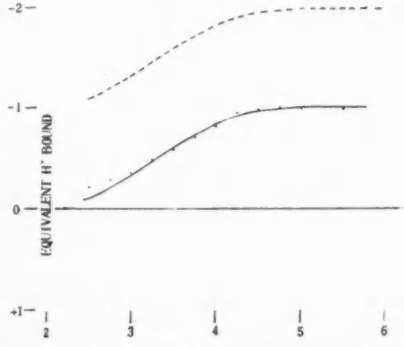


Fig. 1. Hydrogen ion-binding curve for 2 mole periodate oxidation product.
·····: Experimental
——: Theoretical curve for pK'_a 3.3
– – –: Hypothetical curve for structure IIIb.

8) Ref. 5 and H. Musso, *Angew. Chem.*, **68**, 313 (1956).
9) T. V. Parke and W. W. Davis, *Anal. Chem.*, **26**, 642 (1954).
10) The titration was carried out only in the region of pH 2.3 to 6, since the end point was obscured in the other pH regions by the effect of the slightly excess periodic acid (pK_{a_1} at 1.64, pK_{a_2} 6.92 : N. V. Sidgwick, "The Chemical Elements and their Compounds", Vol. II, p. 1237 (1950), Oxford) and the buffering action of the formed ammonium chloride. The inflection at 2.1 was obtained by extrapolation.
11) Inasmuch as the side chain aminoethanol structure is destroyed by the first mole of periodate, it makes no difference whether the structure is I or II. The same applies to III and IV.

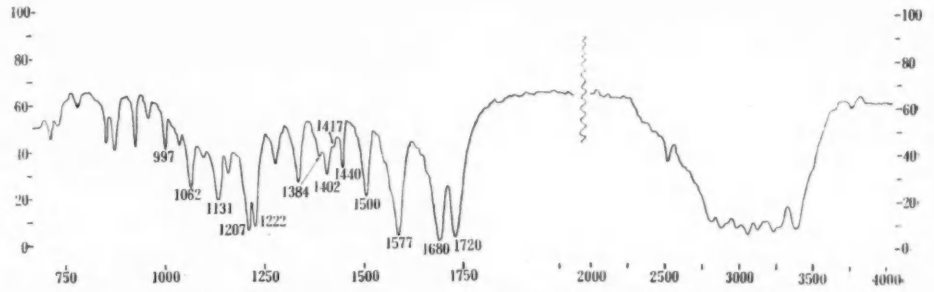


Fig. 2. Roseonine·2HCl (1.1 mg./300 mg. KBr): 1720 (COOH), 1680 (G—I)*, 1577 (G—II, NH_3^+)*,**, 1500(NH_3^+)**

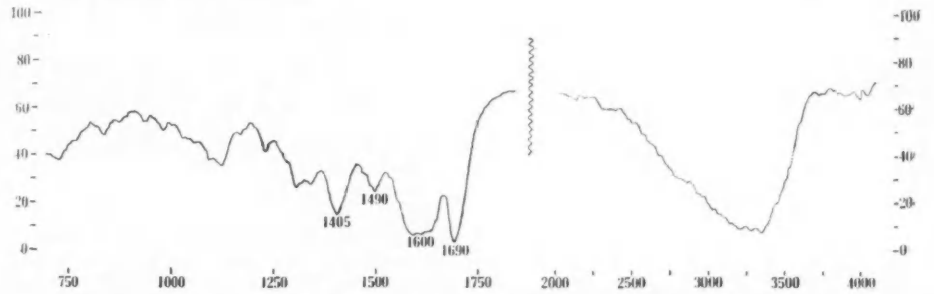


Fig. 3. Free roseonine (crude) (KBr): 1690 (G—I)*, 1600 (NH_3^+, G—II, COO^-)*,**, 1490(NH_3^+)**

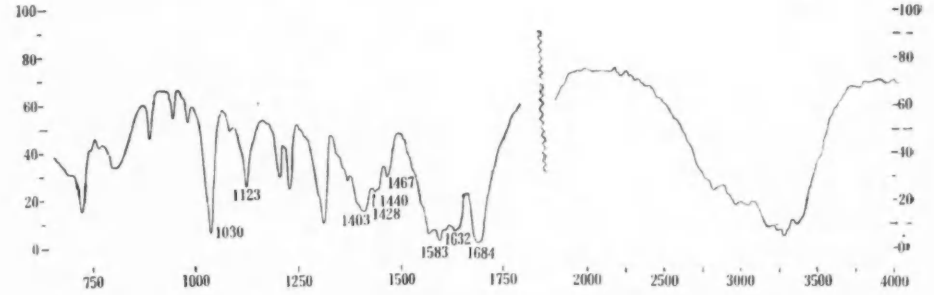


Fig. 4. Roseonine Diol (1.0 mg./300 mg. KBr): 1684 (G—I)*, 1632 (COO^-), 1583 (G—II)*

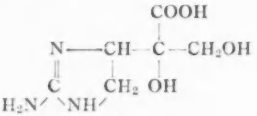
* cf. Guanidinum I and II band, Goto, Nakanishi, Ohashi, preceding paper.
** cf. Nakanishi, Goto, Ohashi, This Bulletin, **30**, 403 (1957).

pK'_a values of 2.1 and 3.3, differentiation may be made by taking into account the hydrogen ionbinding curve for the two-mole oxidation product (Fig. 1). It is to be noted that since the dihydrochloride was used as the starting material, structure Ib possesses one hydrogen ion-releasing carboxyl group, and structure IIIb possesses two such groups; the entire titration curve, in either case, should thus lie *above* the zero line. The incompatibility of structure IIIb with Fig. 1 may be inferred as follows. First, assuming 3.3 to be the first pK'_a value, the second dissociation constant arising from the second carboxyl group must then lie somewhere above 7 since the upper end of the experimental curve ending at about pH 5 as yet shows no sign of a second inflection. The pK'_a2 values for 1,2-*cis*- and *trans*-cyclopentanedicarboxylic acid are at 6.51 and 5.91, respectively[12], and

12) H. C. Brown, D. H. McDaniel and O. Häfliger in "Determination of Organic Structures by Physical Methods", Academic Press (1955), p. 625.

if the additional acid strengthening effect of the guanidinium group is considered, a pK'_a2 value above 7 would be unexplicable. Second, assuming 3.3 to be the second pK'_a value, the first pK'_a should then be below 2 and the experimental curve for the second constant should be that represented by the dotted lines in Fig. 1. Thus it becomes apparent that the observed inflection at 3.3 is due to the carboxylic group of Ib strengthened by the guanidinium group[13], and that roseonine possesses either structure I or II.

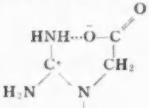
The decision between these two structures, i.e., whether the side chain is an isoserine residue or a serine residue, was further made possible from the infrared spectra. Namely, conversion of roseonine dihydrochloride into the diol V[14] resulted in the appearance of a band at 1467 cm^{-1}



(V)

(Fig. 4) which is absent in the spectra of roseonine dihydrochloride (Fig. 2) or free roseonine (Fig. 3, see experimental for preparation of this compound). This new band can only be assigned to a normal methylene bending vibration, and a new methylene group in the diol could, in turn, only be derived from formula I and not from II. The appearance of a conspicuous absorption at 1030 cm^{-1} in the diol (*prim*-hydroxyl; corresponding to a similar strong band in the spectra of L- and D,L-serine at 1017 and 1036 cm^{-1}, respectively—D-serine was not examined) is also to be noted.

13) The pK'_a of creatine, which like Ib also has a guanidinium group alpha to the carboxyl group is 3.0 at 20°C (Eadie and Hunter, *J. Biol. Chem.*, **67**, 237, 243 (1926)). This slightly enhanced acidity might be explained by structure VI for the zwitterion.



(VI)

14) Obtained by the action of silver nitrite on the dihydrochloride this was the only relatively easily accessible derivative.

Experimental

Potentiometric Titration of Periodate Oxidation Products.—Roseonine dihydrochloride (9.7 mg., 0.037 m.mol.) was dissolved in 3 ml. of water, and to this there was added 3 ml. of an aqueous 0.03 m.mol./ml. solution of sodium metaperiodate. After being kept in an incubator at 35°C, 2 ml. of this mixed solution was pippetted out after 30 minutes and after 20 hours. These solutions were acidified by addition of 1 ml. of 0.0480 N-hydrochloric acid and were titrated with 0.0480 N-sodium hydroxide using a Beckman type-G pH-meter. The pK'_a values were determined by the method of Parke and Davis[9]. The blank curve was obtained by carrying out an identical procedure on a periodate solution to which 3 ml. of water had been added instead of the roseonine-dihydrochloride solution. A parallel reaction mixture was employed for following the periodate consumption; this was measured by conventional techniques. The periodate consumption after 30 minutes was 1.0 mol., and that after 20 hours was 2.1 mol. The titrations were carried out at 11±2°C and not under thermostatted conditions.

Free Roseonine.—This was prepared by dissolving 10 mg. of sodium (0.44 m.mol.) in 1 ml. of absolute ethanol, adding 52 mg. of roseonine-dihydrochloride (0.21 m.mol.), stirring, and leaving overnight. The precipitates of sodium chloride and free roseonine were collected, dried, and pressed into disks. Though the infrared bands were diffuse owing to the presence of sodium chloride, it was sufficient for the purpose of showing the absence of strong absorptions around 1000 cm^{-1} and 1470 cm^{-1} (Fig. 3).

Infrared Absorption Measurements.—The spectra were recorded on a Hilger H 800 double beam instrument equipped with a sodium chloride prism; when necessary the region 1300–1750 cm^{-1} was scanned with a calcium fluoride prism. Potassium bromide disks were used, and the die and the handpress were those supplied by Hilger and Watts, Co. Analytical grade potassium bromide was ground to pass a 200 mesh sieve and dried at 150°C for 24 hours; the samples (ca. 1 mg.) were ground evenly with 300 mg. of this potassium bromide for 5 minutes.

The authors wish to express their gratitude to Professor Yoshimasa Hirata for his guidance and interest in this research and to Mr. Toshio Goto for valuable discussion. We are also grateful for a grant from the Ministry of Education.

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*Structure of Roseothricin A***

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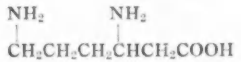
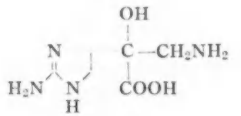
(Received April 25, 1957)

Roseothricin is an antibiotic substance produced by *Streptomyces Roseochromogenus*[1] and has a strong resemblance to streptothricin, streptolin, geomycin etc. Hitherto, two components, β-lysine (I)[2] and roseonine (II)[3], both of which are β-amino acids, have been obtained from the hydrolysate of roseothricin. These two amino acids have also been obtained from the acid hydrolysates of streptothricin[4], streptolin[5], geomycin[6], and racemomycin[7].

Roseothricin is not a simple compound, but consists of three components, roseothricin A, B and C, and these components can be separated by means of paper chromatography, paper electrophoresis or ion exchange chromatography[8]. In order to obtain greater amounts of roseothricin A, the method of ion exchange chromatography was employed: the roseothricin complex was passed through a column packed with Amberlite IRC-50 (Na-form) resin, the column was subsequently eluted with 2% ammonium sulfate solution, and roseothricin A was finally precipitated from the eluate as its phosphotangstate by addition of sodium phosphotangstate solution (cf. experimental part). By this method roseothricin A was obtained in a pure state from the roseothricin complex, and was employed for the following experiments.



(I)



(II)

Components of Roseothricin A.—Acid hydrolysis of roseothricin A with 6 N hydrochloric acid at 100° afforded two ninhydrin positive substances, β-lysine and roseonin, but during the hydrolysis considerable amounts of insoluble black resinous substances were produced[3]. Roseothricin A was therefore hydrolysed with 6 N hydrochloric acid at 50° for 11 days in nitrogen atmosphere, the hydrolysate was passed through a column of Dowex 50 (H-form) and the column was eluted with hydrochloric acid. The results are shown in Fig. 1.

Further hydrolysis of compound B afforded compound A and roseonine, and of compound C afforded β-lysine in addition to the two substances. Thus β-lysine, roseonine, compound A, carbon dioxide and ammonia resulted from the hydrolysis of roseothricin A.

Compound A is a new substance, properties of which are as follows: colorless crystals, dec. p. 145—155°, it shows positive ninhydrin, Fehling, Elson-Morgan, triphenyl tetrazolium chloride, and Tollens reaction and is, in these respects, similar to glucosamine. Paper chromatography: buthanol: acetic acid: water (4 : 1 : 1) R_f. 0.33 (glucosamine [G] 0.33); collidine 0.44 (G. 0.43); phenol : 1% ammonia (concd.) 0.74 (G. 0.70); ethyl acetate : pyridine : water (2:1:2) 0.86 (G. 0.87); methyl ethyl ketone 0.03 (G.

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** A communication (the part of compound A) has appeared in this Bulletin, **30**, 725 (1957).

1) S. Hosoya, M. Soeda, N. Komatsu, S. Imamura, M. Iwasaki, Y. Sonoda and K. Okada, *Jap. J. Exptl. Med.*, **20**, 121 (1949); *J. Antibiotocs*, **3**, (4), 217 (1950).

2) E. E. van Tamelen and E. E. Smissman, *J. Am. Chem. Soc.*, **75**, 2031 (1953).

3) K. Nakanishi, T. Ito, M. Ohashi, I. Morimoto and Y. Hirata, This Bulletin, **27**, 539 (1954); *J. Am. Chem. Soc.*, **76**, 2843 (1954); This Bulletin **30**, 725 (1957).

4) H. E. Carter, R. K. Clark, Jr., P. Kohn, J. W. Rothrock, W. R. Taylor, C. A. West, G. B. Whitfield and W. G. Jackson, *J. Am. Chem. Soc.*, **76**, 566 (1954).

5) E. E. Smissman, R. W. Sharpe, B. F. Aycock, E. E. van Tamelen and W. H. Peterson, ibid., **75**, 2029 (1953).

6) H. Brockmann and H. Musso, *Chem. Ber.*, **88**, 648 (1955).

7) H. Taniyama and S. Takemura, "Abstracts of Papers", 10th Annual Meeting, Pharm. Soc. Japan, April, 1957.

8) S. Hosoya, M. Soeda, N. Komatsu, N. Hara, Y. Sonoda and R. Arai, *J. Antibiotics*, **4** (5), 314 (1951); *Jap. J. Exptl. Med.*, **20**, 683 (1950); Y. Saburi, *J. Antibiotics*, Ser. B **6** (8), 402 (1953).

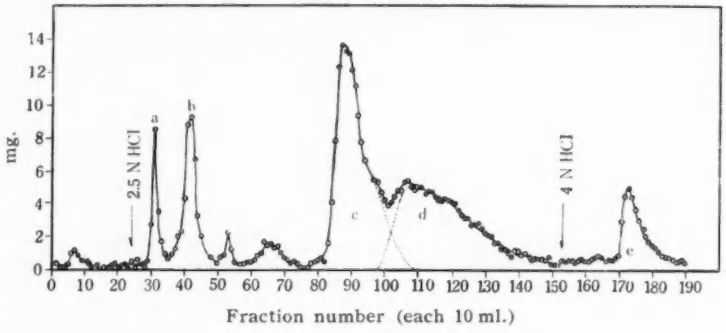


Fig. 1.

Four hundred mg. of roseothricin A HCl salt was hydrolysed to afford 470 mg. of the hydrolysate.

Tube number	Yield	
1—27	9 mg.	
a. 28—34	18 mg.	compound A
b. 35—50	45 mg.	ammonium chloride
51—80	23 mg.	(negative ninhydrin test)
c. 81—100	153 mg.	125 mg. β-lysine. 2HCl 28 mg. roseonine. 2HCl*
d. 101—138	129 mg.	compound B
139—169	21 mg.	
e. 170—190	40 mg.	compound C
Total	438 mg.	(recovery 93%)
	25 mg.	CO_2 (as 111 mg. $BaCO_3$)

* It was calculated from the periodate consumption of fraction C.

0.03). Compound A was scarcely distinguishable from glucosamine by means of paper chromatography using several solvent systems, but there was a small difference between their infrared spectra (Fig. 2). Compound A could not be analysed because of its very minute quantity and of the difficulty obtaining it in a crystalline state. However, according to the analysis and the periodate consumption of compound B, it was shown that compound A was neither a pentosamine nor a branched chain hexosamine, but a straight-chain hexosamine. Recently a hexosamine[9] (m. p. 152—162° dec.) has been isolated from streptothricin and streptolin B, and its structure elucidated as 2-amino-2-deoxy-α-D-gulose (α-D-gulosamine), which is a stereoisomer of glucosamine. Therefore, although no direct comparison between the two has as yet been made, it may be safe to regard compound A as being identical with gulosamine (III).

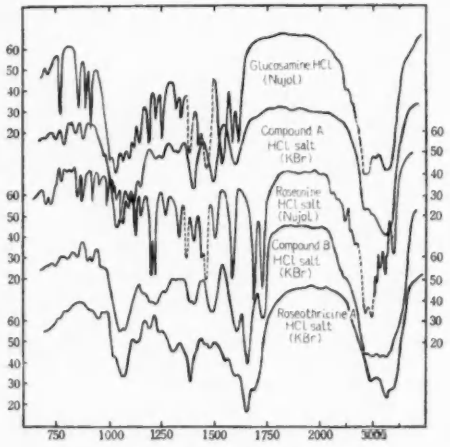
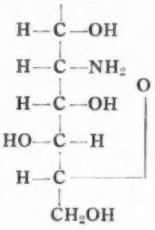


Fig. 2

Structure of Compound B.—Compound B was 2,4-dinitrophenylated incompletely by the method described in the experimental part, and the products were separated by means of counter current distribution. The result is shown in Fig. 3. The two peaks at K=0.16 and 9.0 suggest that compound B has two amino

9) E. E. van Tamelen, J. R. Dyer, H. E. Carter, J. V. Pierce and E. E. Daniels, *J. Am. Chem. Soc.*, **78**, 4817 (1956).



(III)

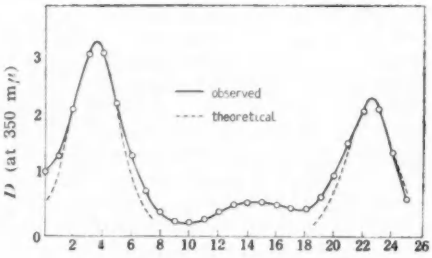


Fig. 3.

groups capable of being 2, 4-dinitrophenylated. The product of complete DNP-ation of compound B has an $E^{1\%}_{1cm}$ value of 306 at 350 mμ, and assuming the presence of two amino groups and taking into account the ε value of 15,900[10] (350 mμ) for a single DNP group, the calculated molecular weight of compound B is ca. 700 for

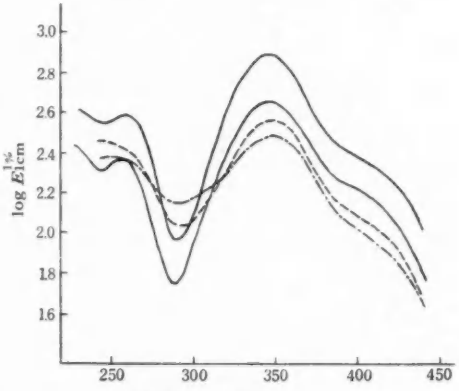


Fig. 4

—— (upper) Di-DNP-β-lysine in tetrahydrofuran $\varepsilon_{350m\mu}=18,000$
—— (lower) DNP-Roseonine in methanol $\varepsilon_{350m\mu}=15,900$
--- DNP-Roseothricin A in T. H. F.
—·— DNP-Compound B in T. H. F.

10) A. R. Batterby and L. C. Craig, ibid., **73**, 1887 (1951). The ε value of 15,900 at 350 mμ is that of DNP-roseonine (roseonine can only be mono DNP-ated).

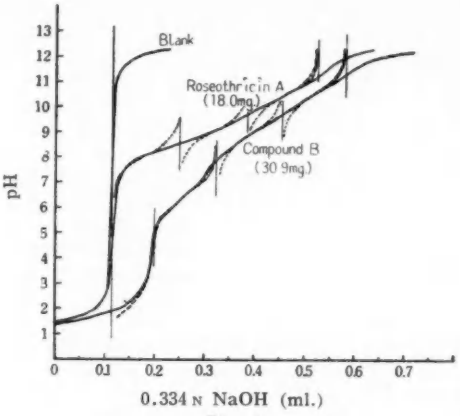


Fig. 5

—— Titration curve
---- Titration curve minus blank
····· Theoretical curve

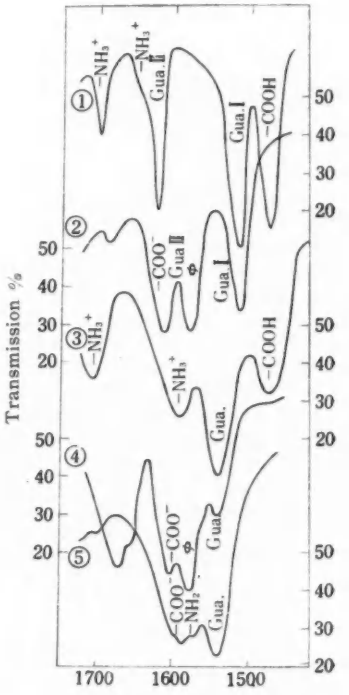


Fig. 6

① Roseonine-2HCl
② DNP- 〃
③ Compound B-HCl
④ DNP-Compound B
⑤ Compound B added NMe_3

the free base (Fig. 4). Though compound B itself could not be hydrolysed without decomposition, roseonine and hexosamine were found in a 1 : 0.8 ratio in the hydrolysate of roseothricin A (Fig. 1). Coupled with the molecular weight (ca. 700) it is assumed that compound B is composed of 2 moles each of roseonine and hexosamine. Compound B, therefore, should have the molecular formula of $C_{24}H_{41}O_{13}N_{10}$ (2 moles each of roseonine and hexosamine minus $3H_2O$) and a molecular weight of 681 as the free base.

Compound B has eight pK_a's: two at 2.4, two at 6.5, two at 8.9 and two at 10.4 (Fig. 5). Since the first two pK_a's at 2.4 undoubtedly arise from the carboxyl groups evident in the IR spectrum (vide infra), it follows that compound B forms a hexa-hydrochloride with the remaining six groups.

Anal. Found; C, 32.65, H, 6.25, N, 16.12 %. Calcd. for $C_{24}H_{41}O_{13}N_{10}$. 6HCl; C, 32.05, H, 5.60, N, 15.57%.

The IR spectrum of compound B showed a band at 1732 cm^{-1}; when trimethylamine was added and the mixture was immediately evaporated to dryness, this band was displaced to ca. 1600, a position corresponding to the absorption of a carboxylate group. Furthermore, this band is present in DNP-compound B, and this is due to the fact that the guanidine portion cannot be DNP-ated and forms an intramolecular salt with this carboxyl group (Fig. 6). The IR of DNP-roseonine led to similar conclusions. This observation coupled with the two pK_a's at ca. 2.4 suggests that the two carboxyl groups of compound B arising from two molecules of roseonine are free, and neither exist in the form of an ester or an amide group. By comparing the pK_a's of compound B with those of roseonine and hexosamine (Table I), it is apparent that the pK_a's at 10.2 originate from the guanidine portion and those at 6.5 and 8.9 originate from amino groups.

Though the IR spectrum of compound B is very similar to the addition spectrum of roseonine and hexosamine (Fig. 2), the spectra of compound B and roseonine differ in the fact that two bands at 1682 and 1581 in the latter are replaced by a single band at 1658 in the former. These two bands of roseonine originate in the N, N'-disubstituted guanidinium group, and if the guanidinium group is N, N', N''-trisubstituted, its IR spectrum shows only one band around 1650 cm^{-1} [11]. The guanidinium groups in compound B is, therefore, symmetrically trisubstituted. Thus the data so far mentioned point to the presence of the following groups in compound B: two amino groups which can be DNP-ated, two carboxyl groups, and two symmetrically trisubstituted guanidine groups. Since hydrolysis of compound B

TABLE I
pK_a' VALUES

Compound	$-COOH$	$-NH_3^+$	Guanidinium$^+$
β-Lysine	3.2	9.5, 10.8	—
Roseonine	2.4	9.3	11.9
Glucosamine	—	7.8	—
Compound B	2.4(2)*	6.5(2)*, 8.9(2)*	10.4(2)*
Roseothricin A	—	8.1(2)*, 9.0(2)*	10.4(2)*

* There are two pK_a's which have the same values; they may not completely coincide but they cannot be distinguished from each other.

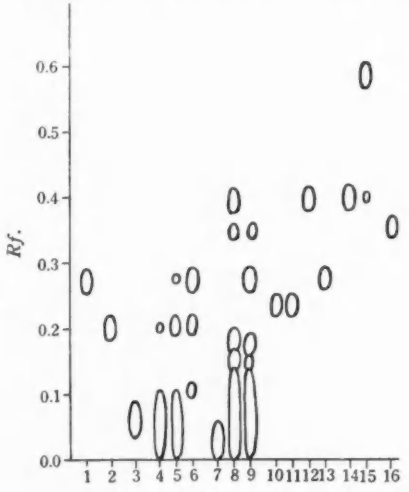
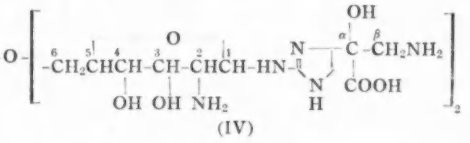


Fig. 7

Solvent system: buthanol : acetic acid : water (4 : 1 : 1). 1. β-lysine. 2. roseonine. hydrolysis of roseothricin A with 0.2 N NaOH at 30°: 3. before hyd. 4. after 1 hour. 5. after 1 day. 6. completely. methanolysis of roseothricin A: 7. with 5% HCl-methanol at 60° for 15 minutes. 8. 20% HCl-methanol, 90°, 3 hrs. 9. hydrolysis of No. 8 with aq. HCl. 10. β-lysine amide 11. treatment of No. 10 with 5% HCl-methanol at 60° for 15 min. 12. treatment of No. 10 with 20% HCl-methanol at 90° for 3 hrs. 13. hydrolysis of No. 12 with aq. HCl. 14. β-lysine methyl ester. 15. treatment of No. 14 with 5% HCl-propanol at 60° for 15 min. 16. compound A.

11) T. Goto, K. Nakanishi and M. Ohashi, This Bulletin, **30**, 723 (1957).

affords roseonine and hexosamine, the only position capable of forming a linkage in the hexosamine moiety is at C_1, which in turn is linked to the guanidine group of roseonine to form a trisubstituted guanidine group (N-glucoside linkage). This is consistent with the fact that compound B gives a positive Elson-Morgan test (glucosamine test) but a negative Fehling test, and the fact that it also gives a positive tetrazolium reaction (semiacetal test) after a longer heating period than that of hexosamine. The strong electron attracting influence of the guanidinium ion probably accounts for the pK_a' (6.5) which is lower than that of hexosamine (ca. 8). The fact that the N-glucoside linkage is more stable under acidic condition than alkaline, is also explicable in terms of this effect. Namely, compound B is extremely resistant towards acid hydrolysis, and no appreciable hydrolysis occurred upon heating for 22 days with concentrated hydrochloric acid at 34°C. It was, however, easily hydrolysed by alkali (1 N sodium hydroxide at 34°C), and gave roseonine (Fig. 7). In this case hexosamine could not be isolated; it was probably decomposed by alkali. As above-menitoned, when compound B was DNP-ated, only two among the four amino groups could be DNP-ated. This partial DNP-ation is probably due to the much slower rate of DNP-ation of the hexosamine residue as compared to the roseonine residue; further DNP-ation of the partial DNP-ated product is thus prevented by precipitation of DNP-compound B. Actually the rate of DNP-ation of glucosamine is less than one fourth of that of roseonine (Fig. 8). Furthermore, it is reported that the amino group of glucosaminide, which has a glucoside linkage, does not react with methyl isothiourea in alkaline condition to form guanidine derivative. This negative evidence may suggest that the glucosidic group affords some protection to the amino group[12]. The positive charge of the guanidinium group in compound B could also retard the reaction between this amino group and the reagent. From these evidences, compound B may be represented by structure (IV).



(IV)

The consumption of periodic acid (6 moles to one mole of compound B, Fig. 9)

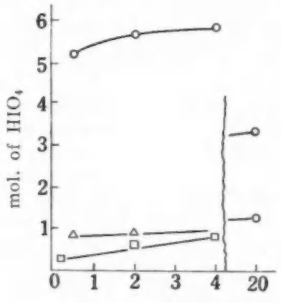


Fig. 9.

suggests that 2-NH_2, 3- and 4-OH, α-OH, and β-NH_2 are all free. Though there exists no direct evidence concerning the linkage between the two hexosamines, ester or amide bands are not observable in the region 1750–1500 cm^{-1}, and this together with the amount of periodic acid consumption suggests that the primary hydroxyl groups and not the secondary hydroxyl groups participate in an ether linkage.

Structure of Roseothricin A.—Roseothricin A was 2,4-dinitrophenylated incompletely, and the products were separated by means of counter current distribution. The result is shown in Fig. 10.

Since there are four peaks (at K=0.026, 0.15, 2.0 and 6.9) roseothricin A should have four amino groups capable of being 2,4-DNP-ated. The complete DNP-ated

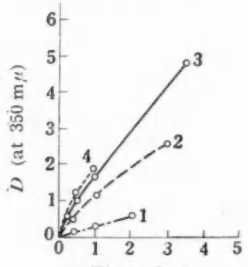


Fig. 8

1 Glucosamine
2 β-Lysine (1/2 eq.)
3 Roseonine
4 Compound B

12) M. Viscontini and J. Meier, *Helv. Chim. Acta*, **35**, 807 (1952).

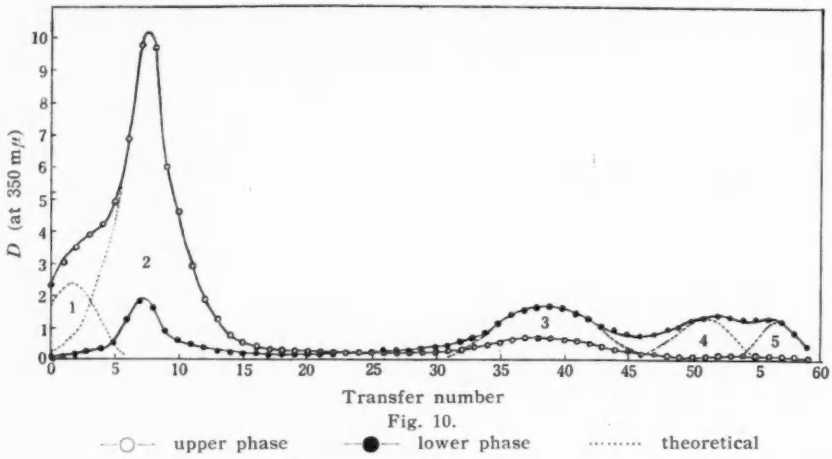
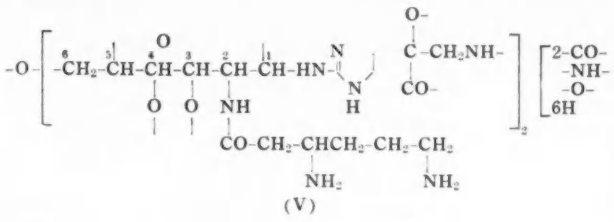


Fig. 10.

product of roseothricin A has an $E^{1\%}_{1cm}$ value of 357 at 350 mμ (Fig. 4). The E value[10] coupled with the presence of four amino groups leads to a molecular weight of ca. 1100 for roseothricin A free base. Acid hydrolysis of DNP-roseothricin A gave di-DNP-β-lysine as the sole product and no derivatives of roseonine or hexosamine were produced. It is thus inferred that there exists two moles of β-lysine in one mol. of roseothricin A and that the four amino groups are all free. Acid hydrolysis of roseothricin A (Fig. 1) afforded 2 mol. of β-lysine (as standard), 1.6 mol. of roseonine, 1.5 mol. of hexosamine, 1.9 mol. of carbon dioxide and 2.2 mol. of ammonia (compound B was divided between roseonine and hexosamine for convenience of calculation). Roseothricin A is, therefore, composed of two moles each of β-lysine, roseonine, hexosamine, and carbon dioxide and one mole of ammonia (the further mole of ammonia was probably produced from roseonine or hexosamine by decomposition, otherwise analytical data do not agree with calculation), and its molecular weight should be 966 as the free base.

Anal. Found: C, 37.97; H, 6.31; N, 16.75%. (no acetyl group). Calcd. for $C_{38}H_{65}O_{16}N_{15}\cdot 6HCl$: C, 37.83; H, 5.93, N, 17.41%.

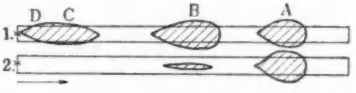
Roseothricin A has six pK_a's: two at 8.1, two at 9.0, two at 10.4 (Fig. 5). This result coupled with the fact that the IR spectrum of roseothricin A is not changed by addition of trimethylamine, suggests that no free carboxyl group is in roseothricin A. Since the amino groups of β-lysine are all free, the four pK_a's at 8.1 and 9.0 must originate from the β- and ε-NH_2 of β-lysine, respectively; the pK_a's at 10.4 are caused by the guanidinium groups. The amino groups of roseonine and hexosamine in roseothricin A are therefore linked to some other groups. When potentiometric titration was carried out in the presence of formaldehyde, the six pK_a's were lowered to two at 3.5, two at 6.1, and two at 8.2. The last pK_a' values (8.2) are higher than that of ordinary amino groups, the pK_a' of which is usually below 7. These data are also consistent with the presence of two guanidinium groups in roseothricin A. Since hydrolysis of roseothricin A with 0.2 N sodium hydroxide at 30°C, afforded first roseonine and subsequently β-lysine, the latter could not be linked to roseonine but should be linked to the hexosamine portion. Heating of roseothricin A with 5% hydrochloric acid-methanol at 60°C afforded neither β-lysine nor its methyl ester, but boiling with 20% hydrochloric acid-methanol afforded β-lysine methyl ester. This behavior was similar to that of synthetic β-lysine amide and not to β-lysine methyl ester (Fig. 7). β-Lysine in roseothricin A, therefore, must be linked to hexosamine through the amino group of the latter. Since roseothricin A did not consume periodate (Fig. 9), at least either the C_3—C_4 or C_1—OH in hexosamine is engaged in a linking (IR spectrum of roseothricin A shows a band at ca. 1717 cm^{-1}, which may be caused by an ester or urethane linkage). We wish to present partial structure (V) for roseothricin A.



(V)

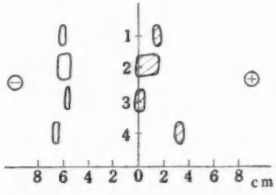
Experimental

Purification of Roseothricin A.—Roseothricin complex (6 g.) was dissolved in 50 ml. of water, and passed through a column of Amberlite IRC-50 (2.5×100 cm tube, 300 c.c. of the resin), after which the column was washed well with water, and eluted with 2% ammonium sulfate solution. The first 10 l. of the eluate did not show any activity on *Bacillus subtilis*, and was discarded. Since the next 20 l. showed strong activity, this fraction was treated with 100 g. of active charcoal. The active charcoal was filtered, washed with water, and added to 250 ml. of 20% acetone-water and an appropriate amount of 1 N hydrochloric acid to adjust the pH to 2, and then filtered. After repetition of this elution procedure for four times, the filtrates were combined, and neutralized to pH 7 by addition of 10% sodium hydroxide solution, and then there was added sodium phosphotangstate solution. The precipitate was centrifuged, washed with water thrice, and dried in a desiccator. This phosphotangstate (ca. 5 g.) was dissolved in 60 ml. acetone and 30 ml. of 2 N hydrochloric acid, filtered from any undissolved substance, and added to 200 ml. of acetone. After being hept overnight in a refrigerator, the precipitate was obtained by decantation, and again dissolved in 10 ml. of water and reprecipitated with 300 ml. of acetone. Finally, the precipitate was dissolved in a small amount of water and freeze dried. Yield ca. 1 g., dec. p. 208–215°. The results of paper chromatography and paper electrophoresis are shown in Fig. 11. The spots were determined by the methods using ninhydrin or *B. subtilis*. The results of electrophoresis suggested that roseothricin had strong basic groups and no acidic groups.



1. Roseothricin complex. 2. Roseothricin A. descending method: solvent; ethanol and 0.5% ammonium sulfate (1 : 1).

Paper electrophoresis



1. Roseonine. 2. Roseothricin complex.
3. Roseothricin A. 4. β-Lysine.
White spot: buffer; 80 ml. of 0.5 M veronal sodium, 2.0 ml. of 2.0N HCl, 72 ml. of 5 M NaCl are diluted to 4 l. (pH 9). 20 mA. 2 hr. Black spot: buffer; 4 ml. of 10% NaOH, 6 ml. of 5 MNaCl are diluted to 500 ml. (pH ca. 12). 20 mA. 4 hr.

Fig. 11.

Hydrolysis of Roseothricin A.—Four hundred mg. of roseothricin A was dissolved in 40 ml. of 6 N hydrochloric acid, and heated at about 50°C under an atmosphere of nitrogen gas which was introduced through two traps containing saturated barium hydroxide solution. After 11 days, the hydrolysate was evaporated to dryness in vacuo, redissolved in a small amount of water and evaporated in a vacuum desiccator containing sulfuric acid and sodium hydroxide. Yield 470mg. The solution of the hydrolysate in 6 ml. water was poured on a column of Dowex 50 (H-form) resin (2×60 cm. tube, ca. 150 c.c. of resin). The column was eluted with water (250 ml.), 2.5 N hydrochloric acid (1300 ml.), and 4 N hydrochloric acid (400 ml.), successively, and the eluates were collected in 10 ml. aliquots by means of a fraction collector. The result is shown in Fig. 1. Each fraction was evaporated to dryness in vacuo below 50°C and weighed. The precipitated barium carbonate in the traps was filtered and weighed.

Partial 2,4-Dinitrophenylations of Compound B and Roseothricin A.—Roseothricin A (83 mg.) and sodium bicarbonate (100 mg.) were dissolved in 10 ml. of 50% ethanol, treated with 5 ml. of 2,4-dinitrofluorobenzene (DNFB 25 mg.) in ethanol (5 ml.), and the solution was allowed to stand overnight at room temperature. A small quantity of yellow precipitate was produced. The solution was acidified with acetic acid, evaporated in vacuo, and then placed in a counter current distributing machine using the solvent system of buthanol: methanol: acetic acid: water (18 : 2: 1 : 19). The result is shown in Fig. 10. The peak No. 5 is 2,4-dinitrophenol. The peak No. 2 was evaporated to dryness, and again 2,4-dinitrophenylated by the same procedure. The result of CCD showed the peaks 2, 3, 4, and 5, except No. 1. The peak No. 2 is much sharper than the theoretical curve; the cause is not elucidated yet.

Partial DNP-ation of compound B was also carried out by the same procedure (9 mg. compound B, 15 mg. sodium bicarbonate 10 mg. DNFB). This result is shown in Fig. 3.

Complete 2,4-Dinitrophenylation of Compound B and Roseothricin A.—Compound B (18 mg.) and sodium bicarbonate (50 mg.) were dissolved in 6 ml. of 50% ethanol, treated with 0.6 ml. of 5% DNFB in ethanol, and incubated at 34°C for two days. The solution was acidified with hydrochloric acid, and the precipitate was

filtered and washed with water, ethanol, and ether, successively. It was dissolved in tetrahydrofuran, and reprecipitated by adding water. Yield 10 mg., m.p. 192–196°C (dec.).

In the case of roseothricin A, the same procedure was also employed (117 mg. roseothricin A, 300 mg. sodium bicarbonate, 6 ml. 5% DNFB in ethanol). The precipitate was dissolved in tetrahydrofuran and reprecipitated with ether. Yield 158 mg., m.p. 210°C (dec. sintered at ca. 200°C).

Hydrolysis of DNP-Roseothricin A.—DNP-Roseothricin A (50 mg.) was dissolved in 5 ml. conc. hydrochloric acid, and incubated in a sealed tube at 37° for 14 days. A yellow precipitate separated gradually after two days. Water was added to the mixture and the precipitates were filtered. The filtrate was almost colorless and contained about 10 mg. of roseonine and no β-lysine (by semi-quantitative paper chromatography). The precipitate (23 mg.) was extracted with ethyl acetate. The ethyl acetate soluble portion was 14 mg.; IR spectra and paper chromatography showed this to be identical with di-DNP-β-lysine. The ethyl acetate insoluble portion (9 mg.) was identical with unchanged DNP-roseothricin A (IR spectra).

Synthesis of Di-DNP-β-lysine and DNP-Roseonine.—β-Lysine dihydrochloride (22 mg.) and sodium bicarbonate (60 mg.) were dissolved in 4 ml. of 50% ethanol, treated with 1 ml. of 5% DNFB in ethanol and allowed to stand overnight. The solution was acidified with hydrochloric acid extracted with ethyl acetate, and the ethyl acetate layer was washed with water, and then extracted with 5% potassium carbonate solution. The aqueous layer was separated and acidified with hydrochloric acid. The precipitate was filtered, washed with water, and recrystallized from a mixture of methanol and acetone. Yellow crystals, m.p. 195–196°C.

Roseonine dihydrochloride (39 mg.) and sodium bicarbonate (90 mg.) were dissolved in 5 ml. of 50% ethanol, treated with 1.5 ml. of 5% DNFB in ethanol and allowed to stand overnight. The reaction mixture was added to 10 ml. of water, acidified with hydrochloric acid, and extracted thrice with ethyl acetate. The aqueous layer was evaporated to 3 ml. and the pH was adjusted to ca. 5 with sodium acetate. DNP-roseonine gradually crystallized from the solution as yellow needles, m.p. 226–229°C (dec.).

Measurements of DNP-ation Rates.—*Reagents*: 0.1% aq. sodium bicarbonate solution, 0.4% DNFB ethanol solution (freshly prepared), 1% hydrochloric acid.

Each sample (10 μ mol.) was dissolved in a mixture of 20 ml. of sodium bicarbonate, 2 ml. of DNFB, and 18 ml. of ethanol at 15°C. At suitable intervals, 5 ml. of the solution was poured into 5 ml. of hydrochloric acid, stop the reaction. The absorption intensity of this solution was measured at 320 mμ and 350 mμ. Calculation was based on the density at 350 mμ and the ratio of the density at 320 to that at 350 mμ. The reaction mixture contained the DNP derivative of the sample used, 2,4-dinitrophenol and unchanged DNFB. 2,4-Dinitrophenol and DNFB had about equal values for the density ratio at 320 mμ/350 mμ, i.e., 2.27 and 2.07, respectively, and 2,4-dinitrophenylamino groups also had equal values (0.50). The absorption density originating from 2,4-dinitrophenol and DNFB could thus be subtracted from that of the reaction mixture. The results are shown in Fig. 8.

Measurements of Periodic Acid Consumption.—*Reagents*: 0.01 M sodium periodate, 0.005 M arsenous oxide, 0.01 M iodine, saturated aq. sodium bicarbonate solution, 10% potassium iodide, and starch solution.

One ml. of compound B solution (9.5 mg. in 10 ml. water) and 2 ml. of 0.01 M sodium periodate were mixed and allowed to stand at 30°C. At suitable intervals the solution was treated with 1 ml. saturate sodium bicarbonate, 2 ml. 0.005 M arsenous oxide, and 1 ml. 10% potassium iodide solution in the order mentioned. After 2 minutes the mixture was titrated with 0.01M iodine using starch solution as indicator. From the difference between the volumes of this titration and blank, the amount of periodate consumption was calculated. By the same procedure, periodate consumptions of the following compounds were measured: roseothricin A (24.6 mg. in 10 ml. water); roseonine dihydrochloride (9.0 mg. in 10 ml.). The results are shown in Fig. 9. The reason that the periodate consumption of roseothricin A increases slowly but linearly and exceeds 3 moles after 20 hours, may be that roseothricin A is gradually hydrolysed during the incubation and consumes periodate.

pK_a' Measurements.—The pK_a's were measured by potentiometric titration in water using Beckmann Model G pH meter.

For example, compound B (18 mg. of its hydrochloride) was dissolved in 1 ml. water (carbon dioxide free), treated with 0.10 ml. 0.40 N hydrochloric acid and titrated with 0.334 N sodium hydroxide. The results are shown in Fig. 5 and Table I.

pK_a' *measurements in the presence of formaldehyde*: As is well known in the formol titration of amino acids, addition of formalin to the solution of primary amino acids causes the pK_a' to drop from a value above 7 to that below 7. For example, β-lysine dihydrochloride (12 mg.) was dissolved in 1 ml. water, treated with 0.3ml. of formalin and 1.0 ml. 0.017 N hydrochloric acid and titrated with 0.268 N sodium hydroxide the results are summarised in the following table:

Compound	pK_{a_1}'	pK_{a_2}'	pK_{a_3}'
β-Lysine	3.2*	4.8	6.9
Roseonine	2.4*	4.9	10.6**
Glucosamine	5.8		
Roseothricin A	3.5(2)+	6.1(2)+	8.2(2)+**

* pK_a' of COOH. ** pK_a' of guanidinium.
+ Cf. footnote of Table I.

Infrared Spectra.—Infrared spectra were measured with a Hilger H 800 spectrophotometer, sodium chloride prism, by the potassium bromide method.

β-Lysine Ester and Amide.—β-Lysine dihydrochloride (3 mg.) was dissolved in 1 ml. of 5% hydrochloric acid-methanol, heated on a water bath for twenty minutes, and then evaporated to dryness. The residue was shown to be β-lysine methyl ester by means of paper chromatography. This ester was dissolved in conc. aq. ammonia and evaporated to dryness to produce β-lysine amide. When β-lysine methyl ester was heated with 5% hydrochloric acid-propanol at 60°C for 15 minutes, β-lysine propyl ester was produced. β-Lysine amide, however, did not produce β-lysine methyl ester by heating with 5% hydrochloric acid-methanol at 60°C for 15 minutes; the ester was first produced by boiling with 20% hydrochloric acid-methanol. The same procedures were applied to roseorthricin A. Roseothricin A produced β-lysine methyl ester by boiling with 20% hydrochloric acid-methanol, but did not produce the ester by heating with 5% hydrochloric acid-methanol at 60°C for 15 minutes. The ester further hydrolysed to β-lysine by boiling in aq. hydrochloric acid. All procedures were protected from moisture.

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A Study on the Molecular Structure of Acrylyl Chloride by Electron Diffraction[1]

By Takeshi UKAJI

(Received May 21, 1957)

Mackle and Sutton[2] proposed a planar structure of acrolein molecule along with other related molecular dimensions, and there is Coulson's discussion[3] regarding the relation between the bond length and the bond order of the compound. Here, the molecular structure of acrylyl chloride was treated by means of electron diffraction method.

Experimental

The preparation of acrylyl chloride was as follows[4]. β-Chloropropionic acid was prepared by passing dry hydrogen chloride through acrylic acid which had been made by hydrolysis of methyl acrylate of commercial grade. It was treated with thionyl chloride at about 80°C to be converted into β-chloropropionic acid chloride. Then the β-chloropropionic acid chloride was gradually dropped onto a catalyser heated to about 200°C which was composed of alumina containing a few per cent. of aluminium chloride and barium chloride. By careful distillation of the chloride, a fraction boiling at 73±1°C was collected.

The electron diffraction photographs were taken in the usual manner using a camera reported in the previous paper[5] (camera distance, ca. 9 cm.; electron wave length, 0.056-0.063 Å, determined by calibration with gold foil). Ten satisfactory photographs were obtained. The diffraction patterns were visually measured up to ca. 90 of q-values.

Analysis

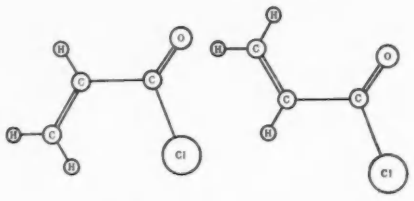
The diffraction photographs were measured by the usual visual method, and the results were shown in Fig. 2 and Table II. The well known formulae for the calculation of the theoretical intensity curves are

$$I_{(q)}=\sum_i\sum_j \frac{Z_iZ_j}{r_{ij}}\exp(-a_{ij}q^2)\sin\frac{\pi}{10}qr_{ij} \quad (1)$$

$$q=(40/\lambda)\sin(\theta/2) \quad a_{ij}=(\pi^2/200)<\Delta r_{ij}^2>$$

where r_{ij} is the distance between the i-th and j-th atom, λ the electron wave length, θ the scattering angle, Z_i the atomic number of the i-th atom, and $<\Delta r_{ij}^2>$ the mean square amplitude of r_{ij}. The thermal vibration term was neglected for the first approximation, i.e. $<\Delta r_{ij}^2>=0$. It was assumed that all the atoms in this molecule were coplanar, and that the distance of C—H and the angle of CCH were 1.08 Å and 120°, respectively. The parameters

1) Presented partly at the Symposium on the structral chemistry held on October 16, 1956, at Nagoya.
2) H. Mackle and L. E. Sutton, *Trans. Faraday Soc.*, **47**, 691 (1951).
3) C. A. Coulson, ibid., **42**, 106 (1946).
4) H. Ueda, T. Sugita and Y. Tanaka, *The Report of the Institute of Synthetic Fiber*, **1**, 635 (1943).
5) T. Yuzawa and M. Yamaha, This Bulletin, **26**, 414 (1953).



trans-form *cis*-form

Fig. 1. Schematic models showing the *trans*- and *cis*-form acrylyl chloride.

C=C and C—C, used in the calculation of theoretical intensity curves, were varied from 1.32 to 1.40 Å and from 1.40 to 1.54 Å in steps of 0.02 or 0.03 Å, and C=O and C—Cl were varied from 1.18 to 1.24 Å and from 1.68 to 1.77 Å with the same increments, while the angles of CCC, CCO, and OCCl were varied from 110° to 130°, from 120° to 130° and from 110° to 130° in steps of 2° or 3°, respectively.

The effect of thermal vibration on the theoretical curves was examined, using the vibration factors a_j listed in Table I, and the curves including thermal terms are shown in the dotted lines in Fig. 2.

A careful comparison was made between the visual intensity curve and the theoretical ones. The curves C_t and C_1 are in the best agreement with the visual one. The curves of several other models also resemble the visual, but they differ from it in the features of the fourth and the fifth maximum and the seventh shelf.

TABLE I

VALUES FOR a_{ij} USED FOR ACRYLYL CHLORIDE MODELS*

Atom Pairs	$a_{ij}\times10^5$
C—H	15.0
C=O	0
C=C	0
C—C	0
C—Cl	12.0
C'···H	20.5
C'···O	5.0
C'···C	8.0
C'···Cl	15.0
C'···Cl	13.0
C''···O (*cis*-form)	20.5
C''···O (*trans*-form)	30.0
C''···Cl (*cis*-form)	28.0
C''···Cl (*trans*-form)	23.5

* All the values are assumed.

The theoretical intensity curves of the *trans*- and the *cis*-form (Fig. 2) remain almost unchanged throughout the features of intensity relation, except the discrepancies of the features of the fourth and the fifth maximum and the seventh shelf. Curves C_1, C_2, and C_3 are the curves of mixtures composed of three parts of the *trans*-form and one part of the *cis*-form, equal parts of the *trans*- and the *cis*-, and one part of the *trans*- and three parts of the *cis*-, respectively.

The fourth and the fifth maximum of the curves A and B are both shifted inward and the seventh shelf has a different shape, while in curve D the fourth maximum changes to a shelf and the sixth minimum shifts outward. Curve E shows that the intensity of the fourth and the eighth maximum is too great in comparison with the fifth and the ninth maximum, and the seventh shelf changes to a peak. Curve F was taken as the typical one for the models which had a greater angle of CCO, the fourth and the eleventh maximum shifting inward and the seventh shelf changing to a small peak. In curves G and H, the features of the fourth maximum and the seventh shelf disagree with the visual ones; in curves I and J the third, the fourth, and the fifth maximum are shifted inward. Curves K and L were made for the purpose of ascertaining the limit of acceptability for C=O distance, both curves being in good agreement with the visual ones in all respects, except the seventh shelf. Likewise, curves O and P were used to show the limit for angle CCC. These curves are rejected because of the disagreement with the visual ones, in the range $q>50$. In curve M, the fourthand the eighth maximum shifted outward, and the tenth maximum changed its position and turned out to be a shelf of the ninth maximum. In curve N, each of the fifth, the sixth, and the eighth minimum, and each of the fifth and the ninth maximum shifted inward; the tenth maximum almost disappeared.

From the above consideration, the models C_t and C_1 are regarded as the most acceptable fits, while the others are all accepted as borderline fits. Nine models are selected from them, and their q_c/q_o values are summarized in Table II.

The radial distribution function for this molecule (Fig. 3) was calculated using the following equation,

$$rD_{(r)}=\sum_{q=1}^{q_{max}} I_{(q)_v}\exp(-bq^2)\sin\frac{\pi}{10}qr \qquad (2)$$

where $I_{(q)_v}$ is the intensity read from the visual curve. The value of b was determined by setting $\exp(-bq^2)=0.1$ at $q=90$.

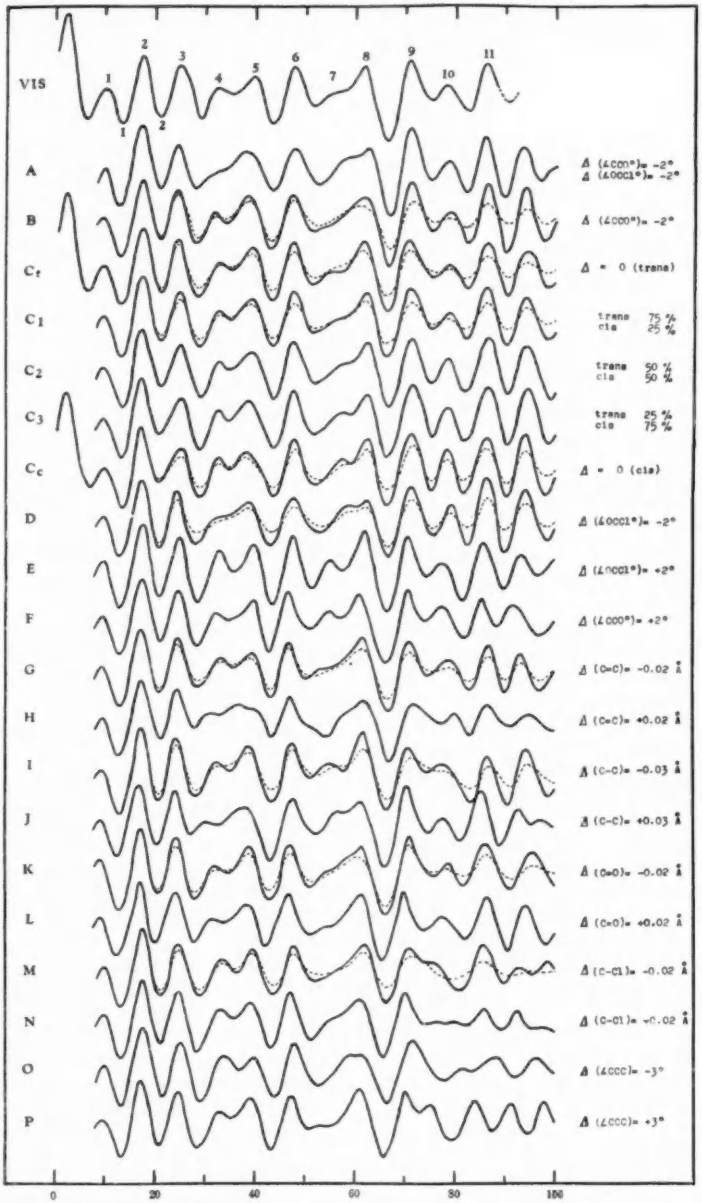


Fig. 2. Visual (VIS) and theoretical intensity curves for acrylyl chloride. The notations on the right hand side of this figure show that the models are deviated from the most probable model, for example, $\Delta=0$ is the most probable model which C—H=1.08, C=C=1.36, C=O=1.20, C—C=1.44, C—Cl=1.74 Å, ∠CCH=120°, ∠CCC =120° and ∠CCO=∠OCCl=125°.

TABLE II
q_c/q_o VALUES FOR ACRYLYL CHLORIDE

Feature		q_0	Model								Wt.
Max.	Min.		B	C_t	C_1	C_c	D	G	I	K	M	
1		10.36	0.923	0.912	0.922	0.965	0.941	0.927	0.946	0.913	0.951	0
	1	13.53	0.957	0.968	0.982	1.009	0.950	0.976	0.979	0.994	0.979	1
2		16.88	1.030	1.031	1.021	1.007	1.028	1.016	1.011	1.036	1.037	1
	2	21.17	0.990	1.000	0.997	0.987	0.992	0.999	0.994	0.999	1.027	1
3		25.07	0.997	0.983	0.987	1.015	0.977	0.981	0.983	0.989	0.997	2
	3	29.15	0.961	0.983	0.995	1.003	0.971	0.983	0.973	0.978	0.997	1
4		32.61	0.986	1.012	1.000	1.000	1.027	1.035	1.004	1.004	1.012	1
	4	36.86	0.922	0.975	0.974	0.943	0.958	0.970	0.963	0.950	0.996	1
5		39.89	0.969	0.991	0.989	0.963	0.974	0.992	0.985	0.985	1.002	2
	5	43.44	0.994	1.014	0.997	1.012	0.997	0.995	1.001	1.000	1.009	2
6		47.58	0.996	0.999	1.000	1.005	1.001	0.984	1.008	1.000	1.010	4
	6	51.14	1.008	1.008	1.007	1.009	1.028	0.987	0.997	1.005	1.026	1
7		(55.15)	—	1.015	0.997	1.042	1.056	0.988	1.015	0.993	—	0
8		61.82	1.000	1.000	1.003	1.009	1.009	1.000	1.003	0.995	1.011	1
	8	66.26	1.003	1.006	1.001	0.996	1.004	1.006	1.004	1.002	1.003	2
9		70.50	1.007	1.000	1.002	1.012	1.001	1.004	1.004	1.009	1.006	4
	9	75.00	1.003	1.003	1.003	1.003	1.010	0.995	0.998	1.009	0.984	1
10		78.38	1.007	1.002	1.002	0.998	1.008	1.010	0.998	1.010	0.963	1
11		86.29	0.998	0.996	0.998	1.001	1.001	1.007	1.008	1.004	0.994	1
Wt. mean			0.993	0.999	0.999	1.002	0.996	0.995	0.997	0.997	1.004	
Wt. mean sq. dev.			0.004	0.003	0.002	0.003	0.004	0.003	0.002	0.003	0.003	

The curve shows that the first and the second peaks correspond to the C—H, C=O, C=C and C—C distances, the third to the C—Cl, the fourth and fifth to the C′···H, C′···O and C′···C, the sixth to the C′···Cl, O′···Cl, C′′···Cl (in the *trans*-form) and C′′···O (in the *cis*-form), the seventh to the C′′···O (in the *trans*-form) and the eighth to the C′′···Cl (in the *cis*-form), respectively. Owing to the complex nature of the peaks, no attempt was made to resolve the distances. It may be noted, however, that the positions determined from the most acceptable model fairly well agree with the peaks observed in this radial distribution curve.

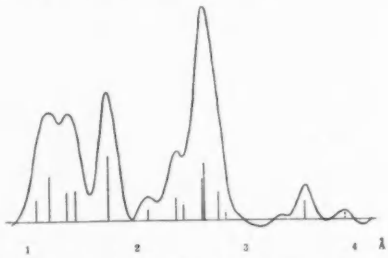


Fig. 3. Radial distribution curve for acrylyl chloride. The most probable interatomic distances are shown by the vertical lines.

In conclusion, the bond distances and bond angles obtained for acrylyl chloride of coplanar configuration are: C=C=1.36 ±0.02 Å, C—C=1.44±0.03 Å, C=O=1.20 ±0.02 Å, C—Cl=1.74±0.02 Å, ∠CCC=120 ±3°, ∠CCO=∠OCCl=125±2°. The *trans*-form is predominant in this molecule.

Discussion of the Results

An electron diffraction investigation of butadiene[6] gave values of 1.35±0.02 Å and 1.46±0.03 Å for the distances of C=C and C—C bond respectivery, and coplanar structure which predominantly had the *trans*-form; these results are in good agreement with the values of 1.36 Å and 1.46 Å in acrolein, respectively. These values agreed with those obtained by C. A. Coulson's theoretical calculation[3] on the C=C, C—C and C=O distances of butadiene, acrolein, and glyoxal. Bond shortening and bond elongation of C—C and C=C bonds in conjugated molecules were plausibly explained by the delocalization of π-electrons. Within the limits of accuracy of this investigation, the circumstances regarding the C=C, C=O distances and the predominance of the *trans* configuration are the same in the case of acrolein.

One of structural studies on acetyl

6) A survey of electron diffraction results through 1949 is found in the tabulation by P. W. Allen and L. E. Sutton, *Acta, Cryst.*, 3, 46 (1950).

chloride[7] shows that the carbon-carbon single bond distance is 1.50 Å and shorter than the normal distance 1.54 Å. Further, the single bond distance between chlorine atom and aromatic nucleus is shorter than the bond length of C—Cl 1.77 Å. From the results of this investigation, it may be considered that the corresponding C—C distance is shorter than that of acrolein, and the C—Cl distance is shorter than that of acetyl chloride.

The values for the C=O bond distance and the angles of CCO and OCCl (1.20 ±0.02 Å and 125±2°, respectively) obtained here are reasonablly compared with those of related compounds shown in reference to the view of the limit of accuracy of the present experiment.

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7) Y. Morino et al., *J. Chem. Soc. Japan Pure Chem. Sec.*, **75**, 647 (1954).

The Electronic Structure of the Hydrogen Fluoride Ion

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(Received August 16, 1956)

In order to throw light upon the hydrogen bond, the author has taken up the $[FHF]^-$ ion that is the simplest one in a theoretical treatment, and has carried out quantum-mechanical calculations for this ion.

As is well known, the hydrogen fluoride ion exists in such ionic crystals as sodium, potassium, and ammonium hydrogen fluorides and consists of the F-H-F hydrogen bond. An X-ray study[1] of potassium hydrogen fluoride has given the F···F distance of 2.26 Å. It has hitherto been a question whether the proton is at the center of two fluorine atoms or not. Peterson and Levy[2] have recently found by the neutron diffraction study that the proton occupies the central position in the linear F-H-F ion.

Based on this fact, the "LCAO SCF MO method" developed by Roothan[3] was applied to the ground state of the $[FHF]^-$ ion and the nature of the F-H-F hydrogen bond was discussed.

Outline of Calculations

The calculation was carried out by taking the eleven atomic orbitals f, f', s, s', σ, σ', π_+, π_+', π_-, π_-' and h into consideration, which stand for 1s, 2s, $2p\sigma$*, $2p\pi_+$ and $2p\pi_-$ orbitals of two fluorine atoms and 1s orbital of the hydrogen atom, respectively. All the electrons were assigned to MO's extending throughout the ion and the interactions between them were explicitely included, while the 1s electrons of two fluorine atoms were treated as the inner-shell of the ion, because of the very small mixing with the valence electrons.

The AO's used were the analytical SCF AO's[4] for neutral fluorine, fluorine anion and hydrogen. The one-center integrals of fluorine atoms were evaluated as the arithmetic mean values of those for neutral fluorine and fluorine anion, using these SCF AO's. This procedure is based on the fact that each fluorine atom in this ion is considered as $F^{-1/2}$. The evaluations of the two-center and the three-center integral using the SCF AO's are, however, very cumbersome and it was, therefore, assumed that the use of Slater-type AO's**

1) L. Helmholz and M. Rogers, *J. Am. Chem. Soc.*, **61**, 2590 (1939).

2) S. W. Peterson and H. A. Levy, *J. Chem. Phys.*, **20**, 704 (1952).

3) C. C. J. Roothan, *Rev. Modern Phy.*, **23**, 69 (1951).

* The $2p\sigma$ and $2p\sigma'$ orbitals are pointed towards the central atom along the molecular axis, respectively.

4) F. W. Brown, *Phys. Rew.* **44**, 214 (1933); Hamano, *Bull. Tokyo Gakugei University* **6**, 9, (1955).

** $h=(1/\pi)^{1/2}\exp(-r)$,
$f=(z_1^3/\pi)^{1/2}\exp(-z_1 r)$,
$s=(z_2^5/3\pi)^{1/2}\exp(-z_2 r)$,
$\sigma=(z_2^5/\pi)^{1/2}\exp(-z_2 r)\,r\cos\theta$,
$\pi_\pm=(z_2^5/\pi)^{1/2}\exp(-z_2 r)\,r\sin\theta\exp(\pm\phi i)$
where $z_1=8.75$, $z_2=2.47$.

TABLE I
SYMMETRY ORBITALS

Symmetry species	σ_g	σ_u	π_u	π_g
Symmetry orbitals	$\sigma_1=f+f'$	$\sigma_5=f-f'$	$\sigma_8=\pi_\pm-\pi_\pm'$	$\sigma_9=\pi_\pm+\pi_\pm'$
	$\sigma_2=s+s'$	$\sigma_6=s-s'$		
	$\sigma_3=\sigma+\sigma'$	$\sigma_7=\sigma-\sigma'$		
	$\sigma_4=h$			

TABLE II
LCAO MO

$K(\sigma_g)=2^{-1/2}\sigma_1$	$K(\sigma_u)=2^{-1/2}\sigma_5$	$1\pi_u=a_{66}\sigma_8$
$1\sigma_g=a_{11}\sigma_2+a_{12}\sigma_3+a_{13}\sigma_4$	$1\sigma_u=a_{44}\sigma_6+a_{45}\sigma_7$	$1\pi_g=a_{77}\sigma_9$
$2\sigma_g=a_{21}\sigma_2+a_{22}\sigma_3+a_{23}\sigma_4$	$2\sigma_u=a_{54}\sigma_6+a_{55}\sigma_7$	
$3\sigma_g=a_{31}\sigma_2+a_{32}\sigma_3+a_{33}\sigma_4$		

instead of the SCF AO's gives satisfactory approximations to these integral values. The greater part of the two-center integrals and a few one-electron three-center integrals*** were exactly calculated. The other two-center and three-center integrals were obtained by Mulliken's approximation[6]. The numerical values of these integrals are given in the appendix.

The $[FHF]^-$ ion is a linear ion belonging to the symmetry group $D_{\infty h}$. The LCAO MO's were chosen so that they belong in sets to irreducible representations of the group $D_{\infty h}$. It is convenient to introduce symmetry orbitals. These were given in Table I with the irreducible representations of the group $D_{\infty h}$.

The LCAO MO's were made up by taking linear combinations of symmetry orbitals, as shown in Table II.

Here the order of increasing number for the MO's within each symmetry species is the order of increasing energy. K refers to MO's made up of inner shell electrons only.

The ground state of this ion is to be $^1\Sigma^+_g$, resulting from configuration $(K(\sigma_g))^2$, $(K(\sigma_u))^2$, $(1\sigma_g)^2$, $(1\sigma_u)^2$, $(2\sigma_g)^2$, $(2\sigma_u)^2$, $(1\pi_u)^4$, $(1\pi_g)^4$.

The total normalized wave function is then built up as an anti-symmetrized product of these LCAO MO's. The requirement that the electronic energy should be an absolute minimum leads to the following matrix equations:

$$(\boldsymbol{H}+\boldsymbol{G})\boldsymbol{a}_i=\varepsilon_i\boldsymbol{S}\boldsymbol{a}_i \qquad (1)$$

As the definitions of these notations are given in detail by Roothan[3] and Mulligan[7], they will not be repeated here. The problem of finding the best LCAO MO's for the ground state thus reduces to the solution of the secular equation (1). This is attained by repeating a self-consistent field procedure.

Results and Discussion

The LCAO SCF MO's of the lowest energy state obtained by the SCF procedure are given in Table III.

TABLE III
LCAO SCF MO

LCAO SCF MO	Orbital energies (eV)
$K(\sigma_g)=2^{-1/2}(f+f')$	—
$1\sigma_g=0.768(s-s')+0.039(\sigma+\sigma')-0.199\,h$	−41.22
$2\sigma_g=-0.133(s+s')+0.533(\sigma+\sigma')+0.464\,h$	−19.20
$3\sigma_g=0.476(s+s')+0.777(\sigma+\sigma')-1.669\,h$	—
$K(\sigma_u)=2^{-1/2}(f-f')$	—
$1\sigma_u=0.708(s-s')+0.082(\sigma-\sigma')$	−41.07
$2\sigma_u=-0.069(s-s')+0.711(\sigma-\sigma')$	−18.46
$1\pi_u=0.502(\pi_\pm-\pi_\pm')$	−16.22
$1\pi_g=0.498(\pi_\pm+\pi_\pm')$	−15.99

Except $3\sigma_g$ orbital, they are filled by two electrons with paired spins in the ground state.

The "LCAO orbital energies" obtained for these LCAO SCF MO's are also shown in Table III. These orbital energies represent good approximations to the ionization energies of the corresponding electron shells. Unfortunately, the experimental values of the ionization energies of the $[FHF]^-$ ion have not yet been obtained. The calculated minimum ionization energy in the present work is 15.99 eV. This value is somewhat lower than that of a fluorine atom (17.42 eV)[8] and that of a hydrogen

*** These integral values were calculated by Oohata's method[5].

5) K. Oohata, *Busseiron Kenkyu*, **50**, 38 (1952).
6) R. S. Mulliken, *J. Chim. Phys.*, **46**, 500, 521 (1949).
7) J. F. Mulligan, *J. Chem. Phys.*, **19**, 347 (1951).
8) C. E. Moore, "Atomic Energy Levels", Vol. 1, Nat. Bur. Standards, Circ. 467, U. S. Department of Commerce, Washington (1949).

fluoride (19.38 eV)****. But this value seems to be too high considering that this ion is an anion. This is considered to be caused by the increasing inter-electronic repulsion compared to fluorine atom and hydrogen fluoride, because this ion is an anion.

It is convenient to adopt an equivalent orbital representation to indicate localized features of the electronic distribution in molecules or ions. The localized equivalent orbitals can be obtained by a unitary transformation of the above MO's. In the case of this ion, the localized equivalent orbitals for the σ-direction can be derived from the four occupied MO's $1\sigma_g$, $2\sigma_g$, $1\sigma_u$, and $2\sigma_u$ as linear sums of these MO's: the two $(b\sigma)$, $(b\sigma)'$ which have a bonding character, the other two $(l\sigma)$, $(l\sigma)'$ which have a lone pair character. These orbitals obtained are given in Table IV.

TABLE IV

EQUIVALENT LOCALIZED ORBITALS

$$(l\sigma) = 0.943s + 0.353\sigma$$
$$(l\sigma)' = 0.943s' + 0.353\sigma'$$
$$(b\sigma) = -0.468s - 0.132s' + 0.809\sigma - 0.139\sigma' + 0.359\,h$$
$$(b\sigma)' = -0.468s' - 0.132s + 0.809\sigma' - 0.139\sigma + 0.359\,h$$

As is seen from this result, the lone-pair orbitals have mainly s-character. On the other hand, in the bonding orbitals, only the σ-electron has a bonding property with the 1s orbital of hydrogen. This indicates that the fluorine orbitals of the F-H bonds have the almost pure p-character.

As a rough estimation of the electron distribution throughout this ion, the polarity of the $[FHF]^-$ ion has been examined. The result from the present calculation indicates that the charge densities of the $[FHF]^-$ ion may be formulated as $F^{-0.78}H^{+0.56}F^{-0.78}$. It would be expected that the polarity of the bond in this ion may be very great, because of the large difference of electronegativity between the fluorine and the hydrogen atom. The above result agrees with this expectations and it indicates that this hydrogen bond is also electrostatic. The special strength of this hydrogen bond is, therefore, considered to be based on the contributions of strong ionic character as well as covalency.

The conclusion obtained may be qualitatively correct, but should not be taken as final. The reasons are as follows: (1) The approximate atomic orbitals were used. (2) Most of the values of energy integrals were approximately evaluated. (3) A configuration interaction was neglected. (4) The MO method was applied to the large distance such as was the case for this ion. For example, the S_{ah} value in this work was 0.2671, while the S_{ah} value using the SCF AO's for fluorine and fluorine anion are 0.3525 and 0.4287, respectively.

In view of these factors, the quantum-mechanical treatment of the hydrogen bonds seems to be more troublesome than that of normal chemical bonds. It is desirable that the improvement of these points should be done in future.

The author wishes to thank Professor K. Kozima and Mr. H. Tsubomura for their kind criticism and Mr. J. Higuchi and Mr. S. Maeda for their helpful advice and discussion.

Appendix

Numerical Tables of Energy Integrals: Values for all but the overlap integrals are given in atomic units (1 atomic unit = 27.204 eV). In the Tables, the energy integrals marked by an asterisk are the approximate values. We shall list the following notations for the many types of energy integrals.

Overlap integrals:

$$S_{pq} = \int \psi_p(1)\psi_q(1)d\tau_1.$$

Kinetic energy integrals:

$$(\psi_p|-1/2\Delta|\psi_q') = \int \psi_p(1)(-1/2\Delta)\psi_q(1)d\tau_1.$$

Nuclear attraction integrals:

$$(A:\psi_p\psi_q') = \int \psi_p(1)(1/r_{a_1})\psi_q'(1)d\tau_1.$$

Coulomb integrals:

$$(pq:r't') = \int \psi_p(1)\psi_r'(2)(1/r_{12})\psi_q(1)\psi_t'(2)d\tau_{1,2}.$$

Exchange integrals:

$$(pr':qt') = \int \psi_p(1)\psi_q(2)(1/r_{12})\psi_r(1)\psi_t'(2)d\tau_{1,2}.$$

Hybrid coulomb-exchange integrals:

$$(pq:rt') = \int \psi_p(1)\psi_r(2)(1/r_{12})\psi_q(1)\psi_t'(2)d\tau_{1,2}.$$

TABLE V

ONE-CENTER INTEGRALS

$(F:ff)$	8.7500	$(ff:pp)$	1.1349
$(F:ss)$	1.3125	$(ss:ss)$	0.7755
$(F:pp)$	1.1456	$(pp:pp)$	0.8061
$\left(f\ -\frac{1}{2}\Delta\ f\right)$	38.2813	$(ss:pp)$	0.7612
$\left(s\ -\frac{1}{2}\Delta\ s\right)$	3.5945	$(p_xp_x:p_zp_z)$	0.7284
$\left(p\ -\frac{1}{2}\Delta\ p\right)$	2.7875	$(fs:fs)$	0.0955
$(ff:ff)$	5.4688	$(fp:fp)$	0.0413
$(ff:ss)$	1.1759	$(p_xp_z:p_xp_z)$	0.0388

**** This value[9] was obtained by the same method as this work. The experimental value for this molecule has not yet been obtained.

9) H. Hamano, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **77**, 985 (1956).
10) R. S. Mulliken, *J. Chem. Phys.*, **46**, 497, 675 (1949).

TABLE VI

TWO CENTER INTEGRALS

S_{sh}	0.3772	$(sh : hh)$	0.1976
$S_{\sigma h}$	0.2671	$(\sigma h : \sigma h)$	0.0767
$(F : hh)$	0.4478	$(\sigma h' : hh)$	0.1851
$(F : sh)$	0.3311	$(\pi h : \pi h)$	0.0157
$(F : \sigma h)$	0.1920	$(ss : sh)$	0.2722
$(H : sh)$	0.2506	$(ss : \sigma h)$	0.1725
$(H : \sigma h)$	0.2671	$(s\sigma : sh)$	0.0349
$(ff : hh)$	0.4474	$(s\sigma : \sigma h)$	0.0711
$(ss : hh)$	0.4338	$(s\pi : \pi h)$	0.0499
$(\sigma\sigma : hh)$	0.4574	$(\sigma\sigma : sh)$	0.2781
$(s\sigma : hh)$	0.0898	$(\sigma\sigma : \sigma h)$	0.1878
$(\pi\pi : hh)$	0.4220	$(\sigma\pi : \pi h)$	0.0068
* $(fh : fh)$	0.0023	$(\pi\pi : sh)$	0.2692
$(sh : sh)$	0.1061	$(\pi\pi : \sigma h)$	0.1648
$(sh : \sigma h)$	0.0773		
$S_{ss'}$	0.0122	$(s\sigma : \pi'\pi')$	0.0307
$S_{s\sigma'}$	0.0176	$(s\pi : s'\pi')$	0.0044
$S_{\sigma\sigma'}$	0.0249	$(s\pi : \sigma'\pi')$	0.0013
$S_{\pi\pi'}$	0.0035	$(\sigma\sigma : \sigma'\sigma')$	0.2478
$(F : ss')$	0.0062	$(\sigma\sigma : \pi'\pi')$	0.2368
$(F : s\sigma')$	0.0096	$(\sigma\pi : \sigma'\pi')$	0.0005
$(F : \sigma\sigma')$	0.0123	$(\pi_+\pi_+ : \pi_+'\pi_+')$	0.2281
$(F : \pi\pi')$	0.0016	$(\pi_+\pi_- : \pi_-'\pi_+')$	0.0003
$(F : s'\sigma)$	0.0081	* $(fs' : fs')$	0.0000
$(F : s's')$	0.2342	* $(f\sigma' : f\sigma')$	0.0000
$(F : s'\sigma')$	0.0320	* $(fs' : f\sigma')$	0.0000
$(F : \sigma'\sigma')$	0.2405	* $(f\pi' : f\pi')$	0.0000
$(F : \pi'\pi')$	0.2310	* $(ss' : ss')$	0.0001
$(H : ss)$	0.4673	* $(ss' : \sigma s')$	0.0002
$(H : s\sigma)$	0.1263	* $(ss' : \sigma\sigma')$	0.0002
$(H : \sigma\sigma)$	0.5155	* $(ss' : \pi\pi')$	0.0000
$(H : \pi\pi)$	0.4431	* $(s\sigma' : \sigma s')$	0.0002
* $(ff : f'f')$	0.2342	* $(s\sigma' : \sigma\sigma')$	0.0003
* $(ff : s's')$	0.2342	* $(s\sigma' : \pi\pi')$	0.0000
* $(ff : \sigma'\sigma')$	0.2405	* $(s\pi' : \pi s')$	0.0000
* $(ff : \pi'\pi')$	0.2310	* $(s\pi' : \pi\sigma')$	0.0000
$(ss : s's')$	0.2342	* $(\sigma s' : \sigma s')$	0.0002
$(ss : \sigma'\sigma')$	0.2405	* $(\sigma\sigma' : \sigma\sigma')$	0.0004
$(ss : s'\sigma')$	0.0320	* $(\sigma\sigma' : \pi\pi')$	0.0000
$(ss : \pi'\pi')$	0.2310	* $(\sigma\pi' : \pi\sigma')$	0.0000
$(s\sigma : s'\sigma')$	0.0088	* $(\pi s' : \pi s')$	0.0000
$(s\sigma : \sigma'\sigma')$	0.0346	* $(\pi s' : \pi\sigma')$	0.0000
* $(\pi\sigma' : \pi\sigma')$	0.0000	* $(s\sigma : ss')$	0.0002
* $(\pi_+\pi_+' : \pi_+\pi_+')$	0.0000	* $(s\sigma : \sigma s')$	0.0003
* $(s\sigma : s\sigma')$	0.0003	* $(s\sigma : \pi\pi')$	0.0001
* $(s\sigma : \sigma\sigma')$	0.0004	* $(s\pi : \pi\sigma')$	0.0000
* $(\pi_+\pi_-' : \pi_+\pi_-')$	0.0000	* $(s\pi : \sigma\pi')$	0.0000
* $(ff : ss')$	0.0089	* $(\sigma\sigma : ss')$	0.0070
* $(ff : s\sigma')$	0.0129	* $(\sigma\sigma : s\sigma')$	0.0101
* $(ff : \sigma s')$	0.0128	* $(\sigma\sigma : \sigma s')$	0.0106
* $(ff : \sigma\sigma')$	0.0183	* $(\sigma\sigma : \sigma\sigma')$	0.0151
* $(ff : \pi\pi')$	0.0026	* $(\sigma\sigma : \pi\pi')$	0.0019
* $(fs : fs')$	0.0001	* $(\sigma\pi : \pi s')$	0.0000
* $(fs : f\sigma')$	0.0000	* $(\sigma\pi : s\pi')$	0.0000
* $(f\sigma : fs')$	0.0000	* $(\sigma\pi : \pi\sigma')$	0.0000
* $(f\sigma : f\sigma')$	0.0000	* $(\sigma\pi : \sigma\pi')$	0.0000
* $(f\pi : f\pi')$	0.0000	* $(\pi\pi : s\sigma')$	0.0100
* $(ss : ss')$	0.0069	* $(\pi\pi : \sigma s')$	0.0096
* $(ss : s\sigma')$	0.0100	* $(\pi\pi : \sigma\sigma')$	0.0137
* $(ss : \sigma s')$	0.0100	* $(\pi_+\pi_+ : \pi_+\pi_+')$	0.0002
* $(ss : \sigma\sigma')$	0.0142	* $(\pi_+\pi_- : \pi_-\pi_+')$	0.0000
* $(ss : \pi\pi')$	0.0020		

TABLE VII

THREE CENTER INTEGRALS

$(F' : hs)$	0.1066	* $(sh : s\sigma')$	0.0023
$(F' : h\sigma)$	0.0954	* $(sh : \sigma s')$	0.0034
* $(H : ss')$	0.0057	* $(sh : \sigma\sigma')$	0.0048
* $(H : s\sigma')$	0.0022	* $(sh : \pi\pi')$	0.0007
* $(H : \sigma\sigma')$	0.0128	* $(sh : s'h)$	0.0614
* $(H : \pi\pi')$	0.0016	* $(sh : \sigma'h)$	0.0442
* $(f'f' : sh)$	0.1285	* $(\sigma h : ss')$	0.0016
* $(f'f' : \sigma h)$	0.0919	* $(\sigma h : s\sigma')$	0.0024
* $(f's : f'h)$	0.0000	* $(\sigma h : \sigma s')$	0.0025
* $(f'\sigma : f'h)$	0.0000	* $(\sigma h : \sigma\sigma')$	0.0354
* $(ss : s'h)$	0.1260	* $(\sigma h : \pi\pi')$	0.0005
* $(ss : \sigma'h)$	0.0900	* $(\sigma h : \sigma'h)$	0.0319
* $(s\sigma : s'h)$	0.0230	* $(\pi h : \pi s')$	0.0000
* $(s\sigma : \sigma'h)$	0.0166	* $(\pi h : \pi\sigma')$	0.0000
* $(s\pi : \pi h')$	0.0000	* $(\pi h : s\pi')$	0.0000
* $(\sigma\sigma : s'h)$	0.1316	* $(\pi h : \sigma\pi')$	0.0000
* $(\sigma\sigma : \sigma'h)$	0.0942	* $(\pi h : \pi'h)$	0.0000
* $(\sigma\pi : \pi'h)$	0.0000	* $(hh : ss')$	0.0053
* $(\pi\pi : s'h)$	0.1231	* $(hh : s\sigma')$	0.0078
* $(\pi\pi : \sigma'h)$	0.0880	* $(hh : \sigma\sigma')$	0.0114
* $(sh : ss')$	0.0023	* $(hh : \pi\pi')$	0.0015

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Measurements of the Amounts of Bound Water by Ultrasonic Interferometer. III. Application to Organic Electrolyte

By Koin ITO and Hazime SHIIO

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Recently many studies have been reported in order to estimate the amount of bound water of solute by measuring the ultrasonic velocity in the aqueous solution. In our laboratory, a general theory was suggested which seemed to give proper result in every kind of solution, and has been applied to solutions of saccharides[1], polyvinylalcohol and its derivatives[2]. In the present paper, an attempt is made to test the theory in the cases of the solutions of several organic electrolytes, and the results are discussed in comparison with those obtained by other methods. Authors chose aminoacids and gelatin as examples of low and high molecular electrolytes, respectively.

As introduced in a previous paper,

$$K=(\beta/\beta_0-V_0)/c\cdot\beta_0/\beta_w=\beta_1/\beta_w\cdot 1/d_1 -(\beta_0/\beta_w\cdot v_0/c-\beta_2/\beta_w\cdot v_2/c) \qquad (1)$$

where the notations are as follows:

V_0=apparent specific volume fraction of total solvent,
i.e., $V_0=(d-c)/d_0$

v_0=apparent specific vol. fraction of the bound water, the density being assumed normal

v_2=specific vol. fraction of the bound water

d=density of solution

d_1=density of solute in solution, i.e., $(1-V_0)/c=1/d_1$

c=concentration of solute in g./cc. of solution

β=adiabatic compressibility of solution

β_0=adiabatic compressibility of solvent

β_1=adiabatic compressibility of solute molecule

β_2=adiabatic compressibility of bound water

β_w=adiabatic compressibility of water.

In the solutions of electolytes, it has been supposed that the bound water might be attached so strongly by the electrostatic force of the ionic radicals of the solute molecule that its compressibility becomes negligibly small. Then, Eq. (1) may be rewritten as

$$K=\beta_1/\beta_w\cdot 1/d_1-\beta_0/\beta_w\cdot v_0/c \qquad (2)$$

Here again, the following procedure previously reported is used to estimate the amount of bound water. When any other bad solvent, though miscible with water, is added to the aqueous solution, the amount of bound water decreases. Therefore, using a bad solvent-water mixture containing a high fraction of the bad solvent, v_0/c has to become small and accordingly Eq. (1) reduces to

$$K=\beta_1/\beta_w\cdot 1/d_1 \qquad (3)$$

Thus, K in such solutions depends on β_1 only. In present cases, ethanol was used as the bad solvent.

Experimental

Commercial chemicals of glycine and α-DL-alanine were purified by recrystallization. Gelatin was dissolved in water and purified by electrodialysis until its electroconductivity decreased to the value of water. Its isoelectric point was pH 4.9.

The adiabatic compressibilities of solution and solvent were calculated by Laplace's relation, $\beta=1/d\mu^2$, from the ultrasonic velocity, μ, which was measured by the ultrasonic interferometer. pH of solution was determined by a glass electrode and pH test paper. The measurements were carried out at 30°C for aminoacids and at 35°C for gelatin.

Results and Discussion

In Table I is shown an example of the

TABLE I

EXAMPLES OF THE MEASUREMENTS (GLYCINE)

Ethanol concn. %	c	d	β/β_0	V_0	K
0.0	0.0819	1.0322	0.8958	0.9520	−0.69
21.1	0.0521	0.9893	0.9539	0.9689	−0.25
33.9	0.0105	0.9517	0.9932	0.9937	−0.05
35.3	0.0105	0.9488	0.9941	0.9935	+0.06
44.2	0.0102	0.9307	0.9938	0.9935	+0.07
48.2	0.0102	0.9229	0.9938	0.9941	−0.03

1) H. Shiio, T. Ogawa and H. Yoshihashi, *J. Am. Chem. Soc.*, **77**, 4980 (1955).
2) H. Shiio and H. Yoshihashi, *J. Phys. Chem.*, **60**, 1049 (1956).

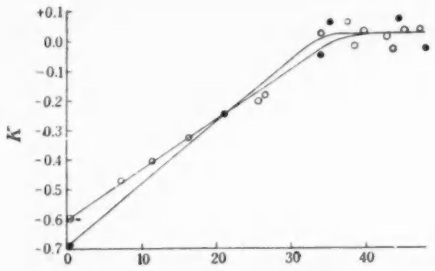

Fig. 1. ●, Glycine; ○, α-DL-alanine

measurements. The results of aminoacids are given in Fig. 1, in which are illustrated the plots of K against ethanol concentration in solvent. The situations are exactly the same as in the cases of glucose and maltose in the previous paper. As the ethanol concentration in solvent increases to some magnitude, K becomes nearly zero. However, in the solutions containing more than about 35 per cent. of ethanol, the solubility of both aminoacids becomes so small that the measurements give inaccurate results. Therefore, the line to which the K curve approaches is not determined exactly, but K may be assumed to be nearly zero in these region. Thus, by extrapolation of this part of the curve to pure aqueous solution, β_1 in aqueous solution is calculated by means of Eq. (3), giving the value approximately zero. Consequently, in such cases of the electrolytic solutions as well as non-electrolytic solutions, the term containing β_1 in Eqs. (1) and (2) can be assumed to be always zero, and so Eq. (1) becomes

$$K = -(\beta_0/\beta_w \cdot v_0/c - \beta_2/\beta_w \cdot v_2/c)$$

which is similar to the equations proposed by others[3]. This agrees with the fact that when the amount of bound water of the solute of low molecular weight has been calculated from the compressibility difference between solvent and solution, the compressibility of the solute molecule has been ignored by many investigators. Thus, the values obtained by the present procedure are clearly about the same magnitude as those of others[4].

Results of gelatin solution are collected in Fig. 2. As the solvents for gelatin, various ethanol-water mixtures were made by

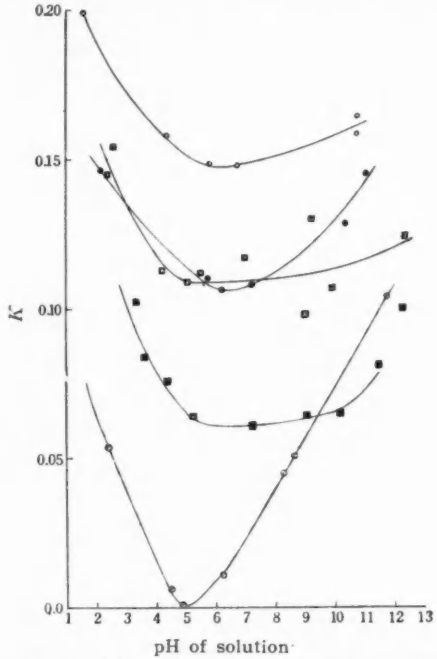

Fig. 2. Ethanol Concn: ⓥ, 0%; ●, 12.9%; ○, 25.0%; □, 34.9%; ■, 42.4%.

adding various small amounts of hydrochloric acid or sodium hydroxide to vary their pH. The fluidity of gelatin solution decreases with the increase in ethanol concentration in the solution. When the ethanol concentration exceeds 45 per cent., gelation takes place in a solution containing several per cent. gelatin even at 35°C. It is shown in Fig. 2 that in each ethanol concentration K has the minimum value at a certain pH which is regarded as the isoelectric point of gelatin in each solution. This may be due to the fact that at the isoelectric point, gelatin holds the highest value of the amount of bound water, or, as Miyahara and Shiio have supposed[5], the compressibility of the gelatin molecule acquires a minimum value as a results of the contraction of the molecule by the intramolecular electric field due to its ionic radicals. The plots of K at some definite pH values against ethanol concentration are shown in Fig. 3 by rearranging Fig. 2. The froms of the curves at each pH are similar to each other

3) T. Yasunaga and T. Sasaki, *J. Chem. Soc. Japan Pure Chem. Sec.*, **72**, 87 (1951); Y. Miyahara, This Bulletin, **25**, 326 (1952).

4) Y. Miyahara, This Bulletin, **26**, 390 (1953).

5) Y. Miyahara and H. Shiio, *J. Chem. Soc. Japan Pure Chem. Sec.*, **73**, 265 (1952).

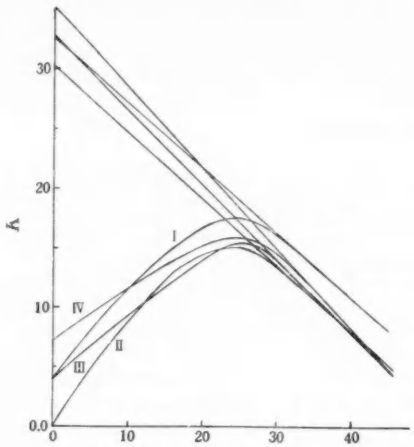

Fig. 3. I, pH 3; II, pH 5; III, pH 8; IV, pH 10

and to those of dextrine and polyvinylalcohol in a previous paper. Accordingly, it may be expected also in the present case that at the left part of curves, their relatively steep slopes are mainly contributed by the decrease in v_0/c and that at the right part of curves their decreases results from the change in β_1 only. On the basis of these assumptions, the values of v_0/c and β_1 in aqueous solution at various pH are evaluated by means of Eqs. (3) and (1), and given in Table II.

TABLE II
THE RESULTS FOR GELATIN IN AQUEOUS SOLUTION

pH	K	$\beta_1/\beta_w \cdot 1/d_1$	$\beta_1 \times 10^{12}$	v_0/c
3	0.04	0.33	21.	0.29
5	0.00	0.30	19.	0.30
8	0.04	0.33	21.	0.29
10	0.07	0.35	22.	0.28

From these values it may be seen that β_1 increases gradually with the deviation of pH from the isoelectric point, while v_0/c changes very little. Consequently, the variation in K in aqueous solution caused by pH change is mainly due to the change of the compressibility of the gelatin particle. The above values of v_0/c agree with the generally assumed values of proteins, and the behavior of v_0/c with pH change agrees with the fact reported by Weber et al.[6].

The hydration of gelatin has been evaluated by Passynski[7]. He proposed an approximate equation to calculate the amount of hydration from the compressibility difference between solvent and solution, which was similar to the present ones, and applied it to many solutions. He derived his equation, however, by assuming that when the concentration of solute approached zero, the effect of compressibility of solute could be ignored. But, according to the present procedure, it is shown that K must be also correlated by the compressibility of solute itself, perticularly in high polymer solutions. Consequently, the effect of the compressibility of solute cannot be neglected at the evaluation of the amount of bound water in these procedures. Further more, when the compressibility of solute molecule changes with the variation of composition of solution, the compressibility of solute should be taken into consideration, as the authors have done.

Miyahara has evaluated the compressibility of egg albumin in aqueous solution, giving 13×10^{-12} c.g.s. unit at its isoelectric point by analogical calculation using the date of aminoacids[8]. Jacobson has computed also the compressibilities of natural proteins, the magnitude of which is in the order of 10×10^{-12} c.g.s. unit by his own procedure[9]. In comparison with these values, the present values of gelatin are appreciably larger. This difference may be due to the difference of properties of molecules, that is to say, natural proteins have the rigid three-dimensionally definite structure, but gelatin is a degradation product of some proteins and has not any definite structure, freely extending, somewhat similar to synthetic polymers such as polyvinylalcohol.

To calculate the values of v_0/c and β_1 of gelatin strictly, however, the facts that there are several interactions between the components of solution must be taken into account. The change of pH of the solution caused by the buffer action of gelatin might also be well considered.

It may be concluded that the present procedure holds not only for the solutions of non-electrolytes but also for those of electrolytes and that the values of the amount of bound water and the compressibility of solute are always separately obtained.

6) H. H. Weber and D. Nachmannsohn, *Chem. Abst.*, **23**, 1917 (1929).

7) A. Passynski, *Acta Physicochim. U. R. S. S.*, **3**, 779 (1935); **22**, 137 (1947).

8) Y. Miyahara, This Bulletin, **29**, 742 (1956).

9) B. Jacobson, *Arkiv För Kemi*, **2**, 177 (1950).

Summary

1. The previously introduced theory on the amount of bound water and the compressibility of solute has been tested with the solutions of organic electrolytes.
2. The proposed procedure was found to be satisfactory and gave separately the values of the amount of bound water and the compressibility of solute in each case. These results appear to be proper compared with others.
3. The compressibility of the solute could be neglected in aminoacid solutions by the same reason as in the case of glucose where the solute is of low molecular weight.
4. It is shown that the amount of bound water of gelatin varies very little as pH of solution goes apart from the isoelectric point where the compressibility of gelatin molecule has a minimum value, as had been expected.

The authors wish to express their sincere thanks to Professor I. Sano and Assist. Professor Y. Miyahara for their kind encouragement and discussion throughout this work.

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Studies on the Mechanism of Eder's Reaction. II. On the Properties of the Active Oxalic Acid in Manganese (III and VII)-Oxalate Reaction Systems

By Shukichi SAKURABA and Syozo IKEYA

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Introduction

The reduction of mercury(II) chloride by oxalate according to so-called Eder's reaction,

$$2HgCl_2+C_2O_4^{2-}=Hg_2Cl_2+2Cl^-+2CO_2$$

can be induced by addition of very small amount of strong oxidizing agents such as potassium permanganate, manganese dioxide, and the manganese(III)-oxalate complexes.

In these induced reactions, an active oxalic acid, which appears to be formed by the decomposition* of manganese(III)-oxalate complex, is usually regarded as one of the chain propagating intermediates[1,2]. The active oxalic acid is very reactive and is able to reduce mercury(II) chloride; for its formula, CO_2^{-}[3], $C_2O_4^{-}$[2,4] or $Mn\begin{matrix}O-C=O\\ \dot{}\\ O-\dot{C}=O\end{matrix}$[5] has been conveniently used to account for the reaction mechanism. Although the chemical formula of the active oxalic acid is not known as yet, its presence was futher confirmed by the fact that when the manganese(III)-oxalate complex was decomposed in the air, hydrogen peroxide was formed in the solution.

Weiss[2] and Abel[6] explained the formation of hydrogen peroxide as follows: the O_2^- ion is produced by the reaction between the active oxalic acid, CO_2^- or $C_2O_4^-$, and the dissolved oxygen. Launer[3] showed that the net rate in the decomposition of manganese(III)-oxalate complex is decreased by oxygen, and he proposed the following mechanism:

$$CO_2^-+O_2=O_2CO_2^-$$
$$O_2CO_2^-+Mn^{2+}+2H^+=Mn^{3+}+CO_2+H_2O_2$$

In these mechanisms, the regeneration of the manganese(III) ion and the formation of hydrogen peroxide are involved.

* In its nature, this is an auto-reduction of manganese(III) complex to manganese(II) salt; those reactions that follow are the same.

1) C. H. Cartledge, *J. Am. Chem. Soc.*, **63**, 906 (1941).
2) J. Weiss, *Discussions Faraday Soc.*, No. **2**, 188 (1947).
3) H. F. Launer, *J. Am. Chem. Soc.*, **55**, 865 (1933); H. F. Launer and D. M. Yost, ibid., **56**, 2571 (1934).
4) H. Taube, ibid., **69**, 1418 (1947); ibid., **70**, 1216 (1948).
5) F. R. Duke, ibid., **69**, 2885 (1947).
6) E. Abel, *Monatsh.*, **83**, 695 (1952).

Table I

Composition and pH of mixtures used in the experiment

Mixture	$K_3Mn(C_2O_4)_3$	$KMnO_4$	$MnSO_4$	$Na_2C_2O_4$	H_2SO_4	pH
A	0.0025				0.005	2.8*
B			0.0025	0.005	0.003	3.2
C		0.005		0.0175	0.024	3.2*
D			0.005	0.005	0.003	3.2

Numerical values are concentration in mol./l.
* Value after the reaction was completed

As to the inhibitory action of oxygen, however, it has been hardly discussed since Launer's report*.

In our previous report[7], it was described that the photochemical reaction between the manganese(II) ion and oxalate induces Eder's reaction, i. e., the reaction proceeds through an active intermadiate produced by the ultraviolet absorption of manganese(II) oxalate in the solution. This active intermadiate seems to be a long-life radical, $Mn\langle^{O-C=O}_{O-\dot{C}=O}$ or $[Mn^{II}-O-\dot{C}=O]^+$, which will be able to reduce mercury(II) chloride, and react on dissolved oxygen and hydrogen ion to form hydrogen peroxide and manganese(III) complex.

From similarity of the properties, it will be expected that the active oxalic acid formed by the decomposition of manganese (III)-oxalate complex is the same as the above-mentioned active intermadiate. In the study reported here, the properties of the active oxalic acid were examined from a new angle, by means of the spectrophotometric measurement, and the mechanism of the reaction system of manganese (III and VII)-oxalate was presumed.

Experimental

Materials.—All chemicals used in the investigation were the same as described in the first report. Potassium trioxalato-manganese(III) trihydrate, $K_3[Mn(C_2O_4)_3]\cdot 3H_2O$, was prepared by Cartledge and Ericks' method[8].

The solutions used in the absorption measurement were prepared to give the composition shown in Table I, by mixing the known stock solutions.

Mixture A was used to examine the change in the absorption spectra on the decomposition of manganese(III)-oxalate complex, and Mixture C was used on the reaction system of permanganate and oxalate. Mixture B and D are comparative solutions of A and C, respectively, that is, in the final state of reaction, the manganese (II) and the oxalate ion concentrations of A and C are the same as those of B and D, respectively.

Procedure.—The decomposition of potassium trioxalato-manganese(III) in acidic solution (Mixture A) and the reaction between permaganate and sodium oxalate (Mixture C) were carried out in an Erlenmeyer flask which was kept at 8° to 10°C and 20°C with a thermostat, respectively, in the dark room. To avoid the influence of dissolved oxygen in the latter reaction, carbon dioxide or nitrogen gas was passed through the solution in the flask containing sodium oxalate and sulfuric acid for 40 minutes, then the solution was mixed with permanganate solution through which nitrogen gas was previously passed at 20°C. At intervals of appropriate times, a few milliliters of the reaction solution was taken out from the flask, and its absorption spectra were measured by Hitachi model EPU quartz spectrophotometer with 1 cm. cells.

Results

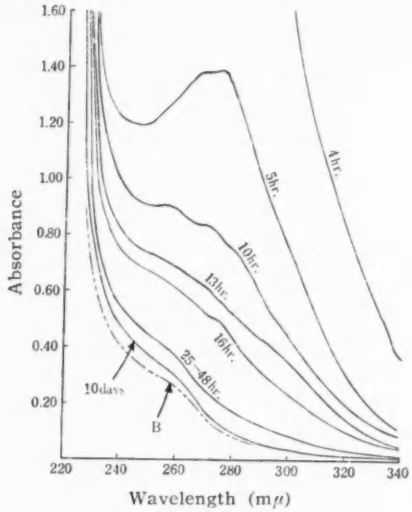
1) The Decomposition of Potassium Trioxalato-Manganese(III) in the Air.—Potassium trioxalato-manganese(III) complex assumes a reddish brown color in water or a neutral solution, and yellow color in an acidic solution, the colors gradually fading away with standing until colorless. It was said that the yellowish color in acidic solution results from the formation of dioxalato-diaquo-manganese(III), $[Mn(C_2O_4)_2(H_2O)_2]^-$ [8].

In the reaction mixture A, it was initially yellow, and its absorption spectra at appropriate times were shown in Fig. 1. After five hours, the solution was pale yellow, and an absorption maximum appeared at about 270 mμ; after ten hours, it became almost colorless, and two absorbance swelling appeared about at 255 and 270 mμ; after thirteen hours, it was colorless, and the absorption swell at 270 mμ almost disappeared. However, the absorbance decreased still more untill it showed slight declining even after ten days. The settled absorption curve had an absorbance swell at about 250 to 260 mμ, and approached to that of the mixture B with a very slow rate. This absorbance swell is due to the absorption of manganese(II)-oxalate complex[7], and that at about 270 mμ, presumably to the absorption of dioxalato-diaquo manganese(III).

* The inhibitory action of oxygen upon the reduction of mercury(II) chloride to mercury(I) chloride has been discussed in numerous reports[1].

7) S. Sakuraba and S. Ikeya, This Bulletin, **30**, 662 (1957).

8) G. H. Cartledge and W. P. Ericks, *J. Am. Chem. Soc.*, **58**, 2061, 2065 (1936).



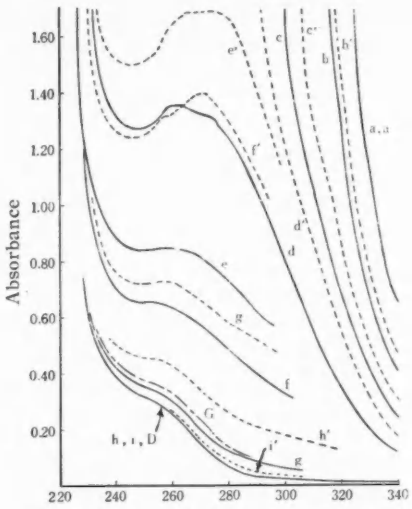
B: Mixture

Fig. 1. Decomposition of potassium trioxalato manganese (III) in the air. (Mixture A)

From these results, it is evident that the declination of absorbance in mixture A signifies the reduction of manganese(III) complex to manganese(II) which is present as manganese(II)-oxalate complex[7] in the solution. After the reaction completed, a large quantity of hydrogen peroxide was detected in the solution*.

2) The Reaction between Permanganate and an Excess of Oxalate.—The reaction between permanganate and oxalate in the composition of mixture C was investigated both in the absence and the presence of dissolved oxygen (in the air).

A start was appointed at the time of addition of the potassium permanganate solution to the oxalate solution. The absorption spectra of the reaction mixture were measured at intervals of an appropriate time, as shown in Fig. 2. The full line is the absorption curve in the absence of oxygen, and the dotted line is that in air. After 50 minutes, the color of the solution changed from violet to purplish red regardless of the presence of dissolved oxygen ; after 70 minutes, it became deep yellow ; after 78 minutes, the color remained unchanged and the absorption curve in the absence of oxygen coincided with that in its presence (curve a, a′ in Fig. 2). After 90 minutes, the color is golden yellow, and the absorption curve in the absence of oxygen became lower than that in its presence (b and b′ or c and c′, etc.). The solutions in the absence and the presence of dissolved oxygen became colorless in curve e (after 130 min.) and curve g′ (after 3 hr.), respectively ; and after about 130 minutes,



—— in nitrogen atomosphere, - - - in air.
a, a′: 78 min. b, b′: 90 min. c, c′: 100 min. d, d′: 117 min. e, e′: 130 min f, f′: 142 min. g, g′: 3 hrs. h, h′: 5hrs. i, i′: 24 hrs. D: Mixture D; G: When g was exposed to air.

Fig. 2. Reaction between $KMnO_4$ and $Na_2C_2O_4$. (Mixture C)

the curves in air showd an absorption maximum at about 270 mμ (curve e′ and f′) and the declining of its curve was much slower than that in the absence of oxygen. The declination of these curves was almost the same as that of manganese(III)-oxalate complex shown in Fig. 1, although the rate for the former was greater than that for the latter*. The pH of the final solution was 3.2, regardless of the presence of the dissolved oxygen, and its absorption curve coincided with that of the mixture D.

These facts suggest that the reaction of permanganate-oxalate proceeds with the formation of manganese(III) on the way to the final state of manganese(II) and carbon dioxide, and that the reduction of manganese(III) complex is retarded by the dissolved oxygen.

When the solution corresponding to the absorption curve g in the absence of oxygen in Fig. 2 was exposed to air and shaken for a few minutes, the curve changed itself to G in Fig. 2**. The same effect was clearly seen, although to a smaller extent, when the solution corresponding to the curve h (after 5 hrs.) and the curve i (after 24 hr.) were exposed to air, but was not seen at all in the mixture D solution. These facts suggest that the manganese(III) complex is produced by an action of the dissolved oxygen. In order to make this suggestion clearer, the absorbance at 270 mμ was plotted against time

* Under this condition of the investigation, the absorption spectrum of hydrogen peroxide was not observed.

* The difference in rate is due to the difference of manganese(II) ion concentration and reaction temperature.

** No effect was exhibited by a violent passing of oxygen gas.

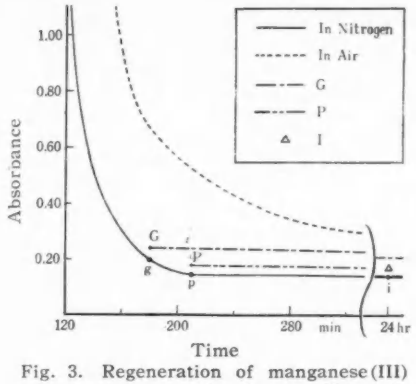


Fig. 3. Regeneration of manganese(III) ion by oxygen; Absorbances 270 mμ in Fig. 2 vis. times.

and shown in Fig. 3. The full line is the curve in the absence of oxygen, the dotted line is that in its presence. When the solutions in the state of g (corresponding curve g in Fig. 2) and p (omitted in Fig. 2) absorbed oxygen in the air, the absorbance increased to G and P in Fig. 3, respectively. On further standing, the slow declination occured along the one and the two dotted lines, respectively. Twenty four hours after standing in the air, the absorbance of solution P almost settled down to that of solution p, but the absorbance of solution G did not entirely to that of solution g. The solution i in Fig. 3 (corresponding the curve i in Fig. 2), which was spectrophotometrically confirmed to be in the final state of the reaction, does not contain manganese (III) salt any more; nevertheless, when the solution was exposed to air, its absorbance increased up to a state of point I, and the solution of i was able to reduce mercury(II) chloride, although the power was weak. Mixture D has the same absorbance with the solution in the state of i (D and i in Fig. 2), however, it does not change the absorbance regardless of the presence of oxygen and has no reducing power towards mercury(II) chloride in the dark at room temperature. Of course, the solutions in which the presence of manganese(III) complex was shown spectrophotometrically (i', g, G, h', etc. in Fig. 2) had also the reducing power towards mercury (II) chloride.

Discussion

From the experimental results above mentioned, the following facts could be stated. The manganese(III)-oxalate complex was formed in the course of the reaction between permanganate and an excess of oxalate, and gradually decomposed to form manganese(II)-oxalate, producing active oxalic acid. The active oxalic acid gives manganese (III) complex and hydrogen peroxide by the action of the hydrogen ion and dissolved oxygen. Hence the reduction of manganese(III) complex will be retarded by the dissolved oxygen. When the manganese(III) complex was completly reduced to manganese(II) in a carbon dioxide or a nitrogen atmosphere and then the solution exposed to air, manganese(III) complex and hydrogen peroxide were reproduced in the solution, therefore, the active oxalic acid appears to survive for a long time.

On the other hand, when the permanganate or the manganese(III) complex was reduced in a solution containing an excess of oxalate ion, its solution possessed reducing power towards mercury(II) chloride, its power was the strongest at pH about 3.2[10].

In the previous report[7], it was stated that an active intermediate was formed by the irradiation of ultraviolet rays on the manganese(II)-oxalate solution, and reacted with the hydrogen ion and dissolved oxygen, forming hydrogen peroxide and manganese(III) complex, and that this formation and the reducing power of the irradiated manganese(II)-oxalate solution towards mercury(II) chloride were most marked at pH about 3.2*.

The active oxalic acid seemingly has the same properties as the active intermediate formed by the irradiation of ultraviolet rays on the manganese(II)-oxalate solution; it seems that the former is to be the same structure as the latter. A possible mechanism can be

$$[Mn(C_2O_4)_2(H_2O)_2]^- \longrightarrow \underset{[I]}{\overset{O}{\overset{\|}{C}}\cdot\!-\!\overset{O}{\overset{\|}{C}}\!-\!O^+ \;\; Mn^{II}} + C_2O_4^{2-} + 2H_2O \quad (1)$$

(or $[Mn(C_2O_4)_3]^{3-} \longrightarrow [I] + 2C_2O_4^{2-}$)

$$[I] \longrightarrow \underset{[II]}{[Mn^{II}\!-\!O\!-\!\dot{C}\!=\!O]^+} + CO_2 \quad (2)$$

$$[II] + O_2 \longrightarrow Mn^{2+} + O_2^- + CO_2 \quad (3)$$

$$Mn^{2+} + O_2^- + 2H^+ \longrightarrow Mn^{3+} + H_2O_2 \quad (4)$$

$$Mn^{3+} + nC_2O_4^{2-} \longrightarrow Mn(III) \quad (5)$$

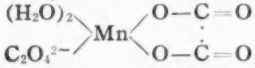
10) S. Sakuraba, *J. Chem. Soc. Japan Pure Chem. Sec.*, **77**, 18, 22 (1956). Experiment was done with the solution in 0.030 mol./l. of $Na_2C_2O_4$, 0.002 mol./l. of manganese(III or VII) and 0.010 mol./l. of $HgCl_2$.

* Experiments were done with the solution in 0.030 mol./l. of $Na_2C_2O_4$, 0.002 mol./l. of $MnSO_4$ and 0.010 mol./l. of $HgCl_2$.

$$[II] + Mn^{3+} \longrightarrow 2Mn^{2+} + CO_2 \quad (6)$$

$$[II] + Hg(II) \longrightarrow Mn^{2+} + Hg(I) + CO_2 \quad (7)$$

The manganese(III)-oxalate complex will be present as trioxalato manganese(III) ion, $[Mn(C_2O_4)_3]^{3-}$, in the solution containing oxalate ions in excess, and as dioxalato-diaquo manganese(III) ion, $[Mn(C_2O_4)_2(H_2O)_2]^-$, in lower oxalate ion concentration. When these ions are subject to auto-oxidation reduction, the active oxalic acid will be produced, and its form was described as CO^-, $C_2O_4^-$ or $CO_2+CO_2^-$, etc., by many investigators. Duke[5] proposed that the electron attractivity of tervalent manganese in a molecule of manganese(III)-oxalate complex is so strong that an electron is donated to the metal ion with simultanious breaking of C—C bond in $C_2O_4^{2-}$ radical, resulting in active complex, to which he gave the formula



From the facts that manganese(II)-oxalate, in the solution as well as in the solid state, is activiated by the light absorption*, and that the properties of this activiated substance are similar to those of active oxalic acid, a formula of the active oxalic acid can be given by [I] as Duke's formula. However, the active intermediate [I] will decompose instantly to form an ionic radical [II] and carbon dioxide. It seems that this ionic radical must have the same formula as the active intermediate formed photochemically from manganese(II) oxalate.

The formation of hydrogen peroxide and the reproduction of manganese(III) complex will be interpreted by steps (3), (4), and (5).

When a sufficient amount of the manganese(III) ion is present in the acidic solution, the active intermediate might be consumed according to step (6), hence, in an acidic solution containing dissolved oxygen, the active intermediate will become extinct by step (3), (4) and (6), and the formation of mercury(I) chloride by step (7) will be also inhibited. On the other hand, in a weak acidic solution containing dissolved oxygen and an excess of the oxalate ion step (1) and (4) will be retarded; consequently, step (6) and (7) will be also inhibited**.

The decomposition of manganese(III) complex in the absence of oxygen will proceed through an equilibrium reaction, $[Mn(C_2O_4)_3]^{3-}+6H^+ \rightleftarrows Mn^{3+}+3H_2C_2O_4$, step (1), (2) and (6), so it will occur more rapidly in an acidic solution. In a weak acidic solution, the concentration of Mn^{3+} is low, and so is that of active radical [I] or [II]; in a strong acid solution, step (6) proceeds more rapidly, because the concentration of Mn^{3+} and [I] or [II] are high, Therefore, contents of the active radical in the solution will scarcely increase more than a limitted extent**.

Through the above consideration, it causes that the decomposition of manganese(III) complex in air is the slowest at pH about 3.2, and the formation of mercury(I) chloride takes place in large quantities at this pH. If the reaction indicated by step (7) is a chain reaction, another intermediate must be considered***.

Summary

1) The active oxalic acid produced in the manganese(III or VII)-oxalate reaction system has the same properties with the activiated manganese oxalate formed by ultraviolet irradiation. These active intermediates are able to reduce mercury (II) chloride and, if oxygen is dissolved in the solution, react with oxygen to form hydrogen peroxide and the manganese(III) ion. These will probably be an ionic radical with a long life that has the same formula.

2) The inhibitory action of oxygen on the decomposition of the manganese(III) oxalate complex can be interpreted by the reproduction of the manganese (III) ion, and it is most remarkable at pH about 3.2.

Department of Appled Chemistry, Faculty of Engineering, Shizuoka University Oiwake, Hamamatsu

* The activiation occurs also by X-ray or γ-ray radiation.

** A kinetic treatment will discussed in the following report.

*** According to Cartledge, the active oxalic acid was regarded as the first necessary chain carrier and the other intermediate was Hg^+ ion[1].

*Studies on the Molecular Structure of Bisacetylacetone-Nickel(II) by Electron Diffraction**,[1]

By Shuzo SHIBATA

(Received May 7, 1957)

Recently, the structures and the properties of metal chelate compounds have attracted considerable attention of many investigators. Acetylacetone chelate is a repesentative of such metal chelate compounds. The author has already investigated the structure of bisacetylacetone-copper(II)[2]. The present paper deals with the structure of bisacetylacetone-nickel(II).

It is interesting to decide whether the arrangement of four oxygen atoms of bisacetylacetone-nickel(II) about the bivalent nickel atom is planar or tetrahedral. The structure of this chelate has already been investigated by magnetic measurements[3] and by absorption spectra[4]. On the basis of the paramagnetic property of bisacetylacetone-nickel(II), Pauling[5] presumed that the chelate has a tetrahedral configuration. However, this evidence has not yet been clarified by other more powerful methods, e.g., the preparation of isomers and the X-ray investigation. In particular, the latter is the most direct method to determine the molecular structure. Recently, the X-ray analysis has been taken by Bullen[6], who showed that there are three nickel atoms in an asymmetric unit, the three atoms being very nearly collinear. Unfortunately he could not definitely clarify the molecular structure of the chelate. The acetylacetone chelate can readily be vaporized without suffering decomposition at a high temperature. Therefore the author has undertaken to apply the electron diffraction method to determine the molecular structure of the nickel chelate in the same way as in a previous study on the copper chelate.

* Presented at Symposium on the Metal Complex Compounds held in November 11, 1952.

1) S. Shibata, M. Kishita and M. Kubo, *Nature*, **179**, 320 (1957).

2) S. Shibata and K. Sone, This Bulletin, **29**, 852 (1956).

3) L. Cambi and L. Szego, *Ber.*, **64**, 2951 (1931).

4) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds", Prentice-Hall, New York, (1952), p. 220.

5) L. Pauling, "Nature of the Chemical Bond", Cornell Univ. Press, Ithaca, (1948), p. 119.

6) G. J. Bullen, *Nature* **177**, 537, (1956).

Experimental

Bisacetylacetone-nickel(II) recrystallized several times from ethanol was furnished by Dr. K. Sone. The anhydrous compound was prepared by sublimation in vacuo. The apparatus[7] used was that already reported by the present author and his collaborators. The sample was vaporized at about 170°C and led through a high temperature nozzle into a vacuum chamber to interfere with an electron beam of about 0.06 Å in wavelength. Non-sector photographs (The camera length was about 11 cm.) were taken in the usual way. Further examination was carried out by s^2 sector photographs (The camera length was about 12 or 28 cm.)

Visual Curve and Radial Distribution Curve

Visual measurements were made of the q values and the intensities of maxima

TABLE I

THE OBSERVED AND THE CALCULATED q VALUES OF THE MAXIMA AND THE MINIMA.

max.	min.	$q_{obs.}$	$q_{calc.}$ (model F)	$q_{calc.}/q_{obs.}$ [a]
	2	7.66	7.7	(1.005)
2		10.20	10.2	1.000
3		14.92	15.0	1.005
4		17.85	17.5	0.980
5		21.26	21.2	0.997
6		24.10	23.7	0.992
7		28.73	29.2	1.016
8		34.40	34.3	0.997*
	10	40.78	40.4	0.991
10		44.07	43.9	0.996*
	11	48.28	48.3	1.000
11		51.44	52.4	1.019
12		58.22	57.6	0.990
13		65.62	65.6	1.000
average of $q_{calc.}/q_{obs.}$				0.998
average deviation				0.007

a) The value of $q_{calc.}/q_{obs.}$ in parentheses was omitted in calculating the average. Those marked with an asterisk were given double weight.

7) M. Kimura, *Chem. Researches*, **9**, 53(1951); S. Shibata and M. Kimura, This Bulletin, **27**, 485 (1954); M. Kimura, K. Kimura and S. Shibata, *J. Chem. Phys.*, **24**, 622 (1956).

and the minima below $q=70$ in the photographs. The measured q values of maxima and minima are given in Table I. The positions of some minima were not measured, because the period of the halos was very short. The visual intensity curve depicted in Fig. 3 was drawn from the measurement of the features of the halos and their positions so as to be represented by the following simplified intensity expression:

$$I(q)=\sum_{i\neq j}(Z_iZ_j/r_{ij})\sin(\pi qr_{ij}/10)$$

Here notations have their usual significances. On the non-sector photographs, the measurements of intensities were considerably difficult in the region of small q (2nd maximum and 2nd and 3rd minima) on account of the intense background of the photographs. Thus the error of this intensity measurement may affect the radial distribution curve. The use of r^2-sector photographs could probably eliminate such a difficulty. The portion of the intensity curve below $q=7$, which could not be observed, was necessary for the calculation of a correct radial distribution curve. Therefore from the observed curve, to which the unobservable part below $q=7$ was properly added, the radial distribution (R.D.) curve was calculated by use of the following formula:

$$rD(r)=\sum_i I(q_i)\exp(-aq_i^2)\sin(\pi q_i r/10)$$

Here the constant a was chosen to be 0.000545 and the summation was carried out up to $q_i=70$. Using the values of some interatomic distances obtained from the resulting curve, the theoretical intensity curves of several models were computed by the above formula. In this case, the ligands were assumed to be planar and to possess a symmetry C_{2v}. This assumption was made throughout in order to simplify this analysis. This assumption would be quite adequate if the contribution of the possible resonance structures of the ligand molecule were taken into account. The calculated $I(q)$ curves indicated that the intensity in the region of small q values (below about 13) was almost invariable, irrespective of whether the configuration of the calculated model was planar or tetrahedral. Accordingly, the observed part was continued with a dotted curve shown in Fig. 3 (upper curve) for the unobservable part of the intensity curve with reference to the calculated curve. The R.D. curve shown in Fig. 1 was calculated from this intensity curve in the same way as before. This curve is almost non-negative. The negative portions in the neighborhood of 3.5 Å and 5.3 Å are attributable to low frequency loops superimposed on this curve, and they seem to be due to the inaccuracy of the intensities for small q values (particularly to the 2nd maximum which was estimated much too high). However, no attempts were made to revise the visual curve because the positions of peaks presumably remain unchanged by this revision. The peaks of this curve could be assigned to the atomic pairs as given in Table II: thus peaks at 1.90, 2.84, 3.20 and

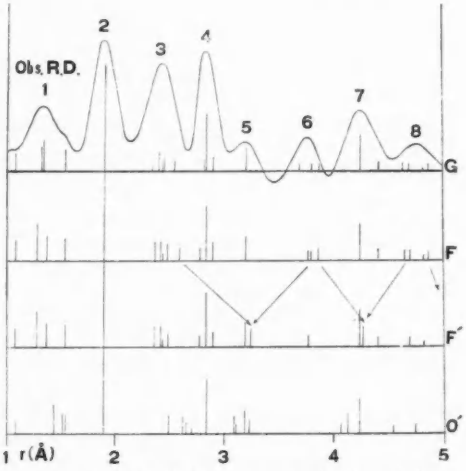


Fig. 1. The R. D. curves of bisacetylacetone-nickel(II). All the atomic pairs of each model are shown by the vertical bars in the computed R.D.

TABLE II
THE POSITIONS AND THE ASSIGNMENTS OF THE PEAKS IN THE R.D. CURVE

peak	distance (Å)	assignment[a]
1	1.33	$C_1O_1(3)$ $C_1C_2(2)$ $C_1C_4(2)$ CH(2)
2	1.90	$NiO_1(10)$
3	2.42	$C_2O_1(2)$ $C_4O_1(2)$ $C_2C_4(1)$ $C_1C_3(1)$ $O_1O_2'(1)$
4	2.84	$NiC_1(5)$ $C_3O_1(1)$ $O_1O_2(1)$
5	3.20	$NiC_2(2)$
6	3.74	$O_1O_1'(1)$ $O_1C_3'(1)$ $C_1C_5(1)$
7	4.25	$NiC_4(3)$ $C_3O_1(1)$
8	4.75	$O_1C_2'(1)$ $O_1C_1'(1)$ $O_1C_5'(1)$ $C_4C_5(0)$

a) These assignments correspond to the best models, G and F. Numbers in parentheses show approximately the relative magnitudes of Z_iZ_j/r_{ij}. The italicized atomic distances vary with the configuration of the chelate.

4.25 Å correspond to Ni–O_1*, Ni–C_1, Ni–C_2 and Ni–C_4, respectively. These four peaks are single or nearly single peaks because in these peaks one atomic pair has a predominant scattering power. Hence, it

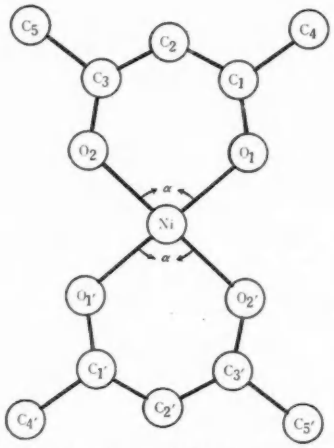


Fig. 2. The shape of the best model and the numbering of atoms.

appears most likely that the values of these interatomic distances are reliable. Furthermore, from the R. D. thus obtained, the reasonable model of the ligand molecule was estimated as follows. When the structure of the ligand is assumed to be as mentioned above, the geometric consideration of the C_2–C_4 and the C_4–O_1 (the 3rd perk at 2.42 Å) and the C_1–C_5 (the 6th peak at 3.74 Å) with the above four distances leads to the model having the following parameters: $\angle O_1NiO_2=92°$, C_1–$C_2=1.37$ Å, C_1–$O_1=1.26$ Å and C_1–$C_4=1.51$ Å. Then the structure of the molecule is determined by the configuration depending on the angle between two ligand planes. Thus the calculation of the theoretical intensity curves for planar and rectangular twisted models showed that the planar model could well reproduce the observed curve but the rectangular model could not. Details will be described below. However it is to be noted for the above assignment that if the molecule takes a rectangular form, the 5th peak can also be assigned to the O_1–O_1' as well as to the Ni–C_2 (this assignment leads to $\angle O_1NiO_2=99°$). Also the height of the 5th peak is fairly low. Therefore the assignment of this peak and the value of the Ni–C_2 distance were carefully taken into consideration.

* The notation "Ni-O_1" also means Ni-O_1', Ni-O_2 and Ni-O_2' by the assumed symmetry of ligand. The situation is the same in the case of other atomic pairs.

Theoretical Intensity Curves

A calculation was made of the theoretical intensity curves for a number of models in order to determine the molecular structure in more detail and to check the accuracy of the interatomic distances obtained from the radial distribution method. However, in a complex molecule such as acetylacetone chelate, a complete determination of the molecular structure by the trial and error method would be very difficult on account of a large number of parameters. Therefore, in order to simplify this task, the present analysis was undertaken by the following assumption on the model. First, the interatomic distances of the Ni–O_1, Ni–C_1 and Ni–C_4, were taken to be equal to the most probable values obtained by R.D. method, i.e., 1.90, 2.84 and 4.25 Å, respectively. (The contribution of these atomic pairs to the total scattering power was about 40%.) Secondly, the structure of the ligands was assumed to be the same as before (C_{2v} symmetry). Then, it follows from the assumptions that molecular models can be determined by four independent parameters for the ligand, i.e., $\angle O_1NiO_2$ (denoted by $\angle\alpha$), C_1–C_2, C_1–C_4 and Ni–C_2 and the angle between the planes of the two ligands. As regards the configuration of the molecule, two cases of a planar form and a rectangular twisted form (including the tetrahedral form) were taken into account. Thus, the theoretical intensity curves were calculated of a large number of carefully selected models (about eighty) with various values for the above parameters. The values of the parameter $\angle\alpha$ was taken over the range from 84° to 110°, that of the C_1–C_2 from 1.32 Å to 1.57 Å. The value of the Ni–C_4 was taken to be 3.20 Å (the value obtained from the R.D.) and 3.10 Å (This smaller value makes the internuclear strain of the ligand smaller in the case of $\angle\alpha$=tetrahedral angle.) The length of C_1–C_4 was taken to be 1.54 Å and 1.51 Å, (because these values are quite reasonable and also because this bond nearly bisects $\angle O_1C_1C_2$ when C_1–C_4 is assumed to take these definite values.) These calculated intensity curves contain the contributions from all the atomic pairs, the pairs pertaining to hydrogen being

ignored except for H–C bondings which were assumed to be 1.08 Å.

The calculation of the intensity curves was performed with the aid of Remington Rand punch cards. When these intensity curves are compared with each other with respect to the variation in the positions and the relative intensities of maxima and minima with the change of parameters, the following facts can be found. In general, the theoretical curves are particularly sensitive to the parameters, $\angle\alpha$ and C_1–C_2. The maxima and these minima sensitive to the parameters are the 8th, 9th, 11th and 12th maxima, and the 10th and 11th minima. The parameter C_1–C_4 does not seriously affect the intensity curve even when this distance differs from 1.54 and 1.51 Å so long as the Ni–C_4 distance does not change. It was also found that the long interatomic distances (greater than 5 Å such as O_1–C_4', C_1–C_1', C_1–C_2', C_1–C_4', C_1–C_5', C_2–C_4', etc.) contribute to the appearance of the 4th and 6th minima. (This contribution is about 7 %.) Some of the intensity curves are shown in Fig. 3.

TABLE III

MODELS FOR THE THEORETICAL INTENSITY CURVES

model	$\angle\alpha$	C_1–C_2 (Å)	C_1–O_1 (Å)	C_1–C_4 (Å)	Ni–C_4 (Å)	configuration
A	88°	1.32	1.23	1.54	3.20	planar
B	90°	〃	1.26	〃	〃	〃
C	〃	1.42	1.20	〃	〃	〃
D	92°	1.37	1.26	〃	〃	〃
E	〃	1.47	1.21	〃	〃	〃
F	94°	1.37	1.28	1.54	〃	〃
F'	〃	1.37	1.28	〃	〃	rectangular
G	96°	1.32	1.34	〃	〃	planar
G_t	〃	〃	〃	〃	〃	〃
$G_{1.51}$	〃	〃	〃	1.51	〃	〃
H	〃	1.42	1.28	1.54	〃	〃
I	〃	1.52	1.23	〃	〃	〃
J	98°	1.32	1.37	〃	〃	〃
K	〃	1.37	1.34	〃	〃	〃
K'	〃	〃	〃	〃	〃	rectangular
L	〃	1.47	1.28	〃	〃	planar
M	100°	1.37	1.37	〃	〃	〃
M'	〃	〃	〃	〃	〃	rectangular
N	108°	1.52	1.40	〃	〃	planar
O	110°	1.52	1.43	〃	〃	〃
O'	〃	1.52	〃	〃	3.10	rectangular (nearly tetrahedral)
P	96°	1.32	1.31	〃	〃	planar
Q	100°	1.37	1.34	〃	〃	〃
Q'	〃	1.37	〃	〃	〃	rectangular
R'	110°	1.52	1.40	1.54	〃	rectangular (nearly tetrahedral)

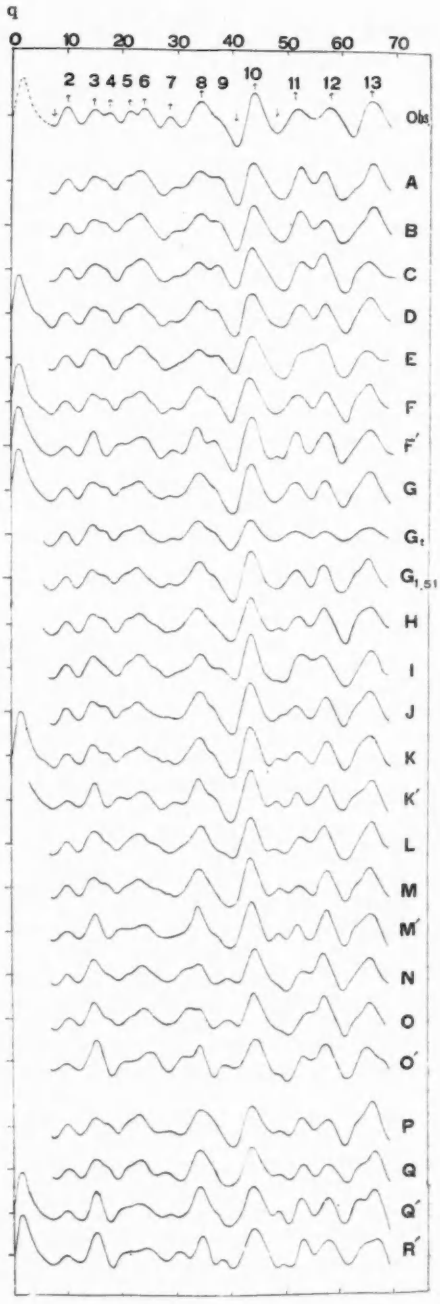


Fig. 3. The observed and the theoretical intensity curves.

The parameters of the corresponding models are listed in Table III. The curve A is unsatisfactory since the 8th maximum is too low and the 9th maximum is too high. Curve B is somewhat unsatisfactory in the same points as for the curve A as well as in the relative intensities of the 11th and 12th maxima. This model gives the lower limit of $\angle\alpha$. The curve C is unsatisfactory, because the 8th and 9th maxima (The observed 9th shelf was so faint that its position could not be measured definitely.) and the 10th minimum (too shallow) are not well reproduced. The curve D is acceptable. This model corresponds to the most probable one estimated from the R.D. curve. The curve E is unacceptable because of the features of the 7th, 8th and 9th maxima, the relative depths of the 10th and 11th minima and the features of the 11th and 12th maxima. The curve F is satisfactory. The curve G is also satisfactory and an agreement with the observed is better than the curve D in regard to the feature of the 9th shelf and the positions of the 11th and 12th maxima and the 11th minimum. The comparison of the curve G with the curve $G_{1.51}$ shows that the parameter C_1–C_4 is sensitive only to the relative heights of the 11th and 12th maxima. The curve H is somewhat unsatisfactory and provides the upper limit of C_1–C_2 since the 7th and 9th maxima are too faint. The curve I with C_1-C_2=1.52 Å is also unsatisfactory because of the feature of the 7th, 8th and 9th maxima and the relative depths of the 10th and 11th minima. The curve J is somewhat unsatisfactory because the relative heights of the 11th and 12th maxima deviate from observations and it provides the upper limit of $\angle\alpha$. The curve K is unacceptable because of the feature of the 11th maximum. The curve L is unacceptable. Model M having $\angle\alpha$ larger than 98° is unacceptable. Model N and O having $\angle\alpha$ almost equal to the tetrahedral angle are definitely unacceptable since they require the intensity curve which is discrepant from the observed curve particularly in regard to the range of q from 30 to 60. In models having large $\angle\alpha$, C_1–C_2 cannot be taken smaller than the above value because C_1–O_1 becomes unreasonably longer than 1.42 Å. The above models are indicated in a parameter chart, Fig. 4. Although a number of intensity curves were also calculated for the corresponding

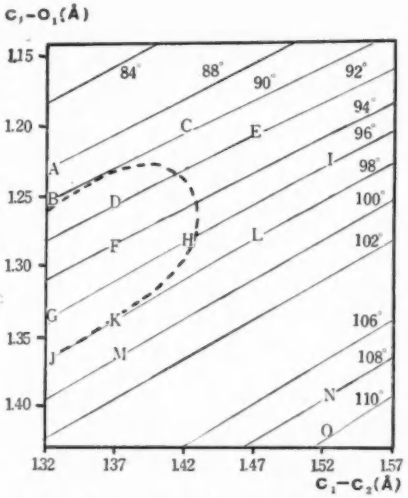


Fig. 4. Parameter chart. This chart shows the case of the parameter Ni–C_2 =3.20 Å. The positions of letters indicate the parameters of the models for which intensity curves are shown in Fig. 3.

models mentioned above with Ni–C_2=3.10Å, these curves did not agree with the observed one. Some of them are shown in Fig. 3. (see curve P and Q.) For example, if the curve P is compared with the corresponding curve G, it is found that the shortening of Ni–C_2 makes the 9th maximum lower and shifts the 11th maximum to a larger q value. In the present analysis the value of Ni–C_4 was also varied, but better agreement could not be obtained. The calculations show that the parameter Ni–C_4 is sensitive only to the relative heights of 5th and 6th maxima. If the molecule takes a configuration with one ligand plane at right angles to the other ligand plane, such as in models F′, K′, M′ and O′ (The last is a tetrahedral model.), in general, the features of the 3rd and 4th (disappearing) maxima and the position of the 5th maximum disagree remarkably with those of the observed curve. It is to be noted that these changes occur in the range below q=30, because in regard to other acetylacetone chelates the configurations of these chelates can easily be determined by only the intensities in this part. Quite similar kinds of behavior are seen in the case of the models having Ni–C_2=3.10 Å such as models Q′ and R′. Therefore, all the rectangular models including the

tetrahedral models are ruled out*.

This conclusion was also confirmed by the computed R.D. shown by the vertical bars below the observed R.D. in Fig. 1. The positions of the bars show the interatomic distances; the heights of the bars are drawn proportional to Z_iZ_j/r_{ij} of the respective atomic pairs. The upper computed R.D. corresponds to the model G which is in good agreement with the observed R.D. as regards the positions and the areas of the peaks. The R.D. F also agrees well with the observed R.D., whereas the computed R.D. F′ is in poor agreement with the observed R.D. with respect to the 5th, 6th and 8th peaks because the ratio of the area of each of these peaks to the sum of the heights of the corresponding bars extremely deviates from the ratio on other peaks. Here oblique arrows connect corresponding atomic pairs. Accordingly the planar model is superior to the rectangular twisted models. The lower R.D. corresponds to the tetrahedral model O′ and the disagreement with the observed R.D. is much enhanced. If the molecule takes the tetrahedral form, the 5th peak in the observed R.D. would rise and the 6th peak would disappear.

It must be mentioned also that the effect of molecular vibration was not taken into account in the above description because it did not seriously affect the features of the intensity curve below $q=70$. This effect was included in the calculation with the following values for root mean square amplitude, $<l_{ij}^2>_{Av}^{1/2}$, 0.07**Å for Ni–O, 0.06 Å for C–C, 0.05 Å for C–O, and 0.08 Å for C–H, by use of the formula:

$$I(q)=\sum_{i\neq j}(Z_iZ_j/r_{ij})\exp(-bq^2)\sin(\pi qr_{ij}/10),$$

where $b=(\pi^2/200)<l_{ij}^2>_{Av}$

As an example, curve G_t is shown in Fig. 3. From the comparison of curve G_t with curve G, it was found that this effect did not affect the positions and the relative intensities of the maxima and the minima except the introduction of simple intensity damping but that this effect produced a slight change in the relative intensities only for the 10th and 11th minima and the 11th and 12th maxima.

* The intensity curves were also calculated for the mixture of the planar and the rectangular models at the ratio of 25~75%, but these curves did not agree with the observed one.

** This value was obtained from a preliminary study on cobalt(II) acetylacetone chelate by the sector-microphotometer method. Also the values for non-bonding atomic pairs were estimated using the data and the calculated values on other compounds.

Results

The computation of intensity curves showed that the general appearance of the the photographs was well reproduced by planar model. In the parameter chart, Fig. 4, the dashed line separates satisfactory models from unsatisfactory ones with due regard to atomic vibrations. The q values of the maxima and the minima for best model are listed in Table I for comparison with those of the observed maxima and minima. From the analysis described above, the molecule of bisacetylacetone-nickel(II) was found to have the planar configuration, with the following parameters: Ni–O_1=1.90 Å, Ni–C_1=2.84 Å, Ni–C_2=3.20 Å, Ni–C_4=4.25 Å (These values were obtained from the R.D. and the correctness of these values were checked by the calculations of the theoretical intensity curves.), $\angle\alpha=94\pm4°$, C_1–C_2=1.37 ±0.05 Å, C_1–O_1=1.30±0.03 Å, and C_1–C_4 =1.51 Å~1.54 Å. Although it is impossible to determine accurately C–C and C–O distances on account of the complexity of the molecule, it is an important conclusion that the chelate has a planar configuration with Ni–O distance equal to 1.90 Å.

Discussion

It is convenient to discuss the results under two headings: (i) bond lengths and bond types, and (ii) stereochemical configuration.

(i) *Bond lengths and bond types.* It must, at first, be mentioned that the observed Ni–O distance of 1.90 Å leads to the radius 1.24 Å for Ni(II) in square configuration with coordination number 4, in contrast with the known radius, 1.39 Å in octahedral configuration, although Pauling[8] estimated the square radius of Ni(II) to be 1.21 Å from the octahedral radius.

The observed Ni–O distance is slightly greater than the sum of the covalent radius of oxygen (0.66 Å) and the metallic radius[9] of nickel with a coordination number 8 (1.22 Å), but is in good agreement with the sum of the covalent radius of oxygen and the metallic radius[9] of nickel with a coordination number 12 (1.25 Å) or the neutral atomic radius[10] (1.24 Å). This may afford an evidence for the Ni–O bond is fairly covalent. On the

8) Ref., 5, chapt. 5.
9) Ref., 5, chapt. 11.
10) N. V. Sidgwick, "The Chemical Elements and their Compounds" Vol. II, Oxford Univ. Press, (1950), p. 1317.

other hand, if Ni–O bond were purely ionic, the distance would be predicted from a simple calculation* to be about 2.2 Å. Such examples are NiO, $NiSO_4 \cdot 6H_2O$, and $NiSO_4 \cdot 7H_2O$[11], in which Ni–O distances have actually been found to be equal to 2.0~2.2 Å. Because no structure of chelates containing an Ni–O bond has yet been studied in detail, the Ni–O bond length observed for bisacetylacetone-nickel (II) cannot directly be compared with other data. The recent data on Ni $(CH_3COO)_2 \cdot 4H_2O$[12] gave about 2.1 Å for Ni–O, but this bond may be considered to be essentially ionic.

Bisacetylacetone-nickel(II) chelate possesses non-ionic properties and a considerable stability. The non-ionic properties of the chelate, i.e., the volatility and the solubility in organic solvents, may naturally be explained by the covalent character of the Ni–O bonds described above. It is noticed that the Ni–O bond length is almost equal to the covalent bond length although the electronegativity difference between Ni and O atoms leads to an ionic character of about 50 per cent. for the Ni–O bond. This is presumably due to the formation of double bond by use of some of the $d\pi$-type orbitals of the nickel atom. On the other hand, the stability of the chelate is attributable to the great strength of dsp^2 hybrid orbitals of nickel atom. This seems to be also due to the contributions of many resonance structures of the ligand molecule which are due to the conjugation among $2p\pi$ orbitals of ligand atoms and $3d\pi$ or $4p\pi$ orbitals of the nickel atom. Further, the fact that the chelate forms the planar rather than the tetrahedral configuration makes more possible the conjugation over the whole molecule consisting of eleven members, resulting in the enhanced stability of the chelate.

(ii) *Stereochemical configuration.* In general, it is well known[13] that the quadricovalent complexes of copper(II) are invariably planar, whereas those of nickel(II) and cobalt(II) may be either planar or tetrahedral depending on the ligand. It is generally found further that with nickel(II) complexes the choice of these configurations is particularly sensitive to the electronegativity of the donor atoms. In other words, Ni(II) complexes with the donor atoms, N or S take a planar configuration and those with O take the tetrahedral one. No planar configuration of the Ni(II) complexes having the donor atoms of O has been found experimentally until the present study was made on bisacetylacetone-nickel(II) chelate.

According to magnetic measurement[1,3], bisacetylacetone-nickel(II) chelate is paramagnetic. From this result, Pauling presumed that Ni(II) has the tetrahedral orbitals which are constructed from sp^3 hybridization. The present result is contradictory to his presumption, although it seems likely that magnetic data do not necessarily exclude the planar form because the promotion of a $3d$ electron to a $4p$ orbit makes the chelate paramagnetic even in $3d4s4p^2$ planar configuration and also the use of $4d$ orbit makes it likewise paramagnetic in $4s4p^24d$ planar configuration as suggested by Huggins[14].

However, the present result that the chelate takes the planar rather than tetrahedral configuration seems to be not surprising because dsp^2 bonds are stronger than sp^3 bonds and moreover the conjugation over the whole molecule in favor of the planar form probably makes the chelate more stable. Some energy may be, of course, required when the Ni(II) takes the electron configuration of $3d4s4p^2$ with paired electrons promoted in one of the $3d$ orbitals or an unpaired electron promoted in one of the $4p$ orbitals. However, such promotion energy will probably be compensated by the stabilization energy acquired by taking this configuration.

It is interesting to note for the above contradiction that the present experiment deals with free molecules at a high temperature, while magnetic measurement and other experiments deal with the chelate in the solid state at room temperature. If the presumption of Pauling is true, it is possible that the configuration of the chelate would vary with the phase change (or temperature**), taking planar in gaseous state and tetrahedral in solid state, because the energy difference between

* Pauling's univalent ionic radii were used after the correction for the ionic valence and coordination number.

11) R. W. G. Wyckoff, "Crystal Structure", Interscience Publishers, New York, Vol. I (1948), Vol. II (1951).

12) J. N. van Niekerk and F. R. L. Schoening, *Acta Cryst.*, **6**, 609 (1953).

13) Ref., 4, p. 278.

14) M. L. Huggins, *J. Chem. Phys.*, **5**, 527 (1937).

** In genaral, the configuration of the chelate is distinguishable by the color of the chelate. Although it is doubtful whether the following is related to the above or not, it is actually observed in experiment that the green color of the chelate was slightly tinged with yellow on heating. (Ref., 5, p. 122).

the planar and the tetrahedral structures is very small. Recently it is found that, for example, bis-N-methylsalicylaldimine-nickel(II)[15] behaves in a similar manner although all such chelates hitherto reported have both N and O atoms as donors. In order to clarify the above suggestion and the discrepancy between the conclusions from the magnetic data and the electron diffraction data on bis-acetylacetone-nickel(II), the following two investigations are very desirable; the complete determination of the structure of the chelate on single crystals by X-ray method*** and the measurement of the magnetic moment of the chelate in gaseous state. The latter will also give the information on the electronic state of Ni(II) atom which has the planar orbitals.

The author wishes to thank Professor M. Kubo for his valuable advice and encouragement, and Professor Y. Morino for his kindness in providing facilities for the numerical computations by punch cards. The author also expresses his deep appreciation to the Ministry of Education for supporting this research.

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15) S. Fujii and M. Sumitani, *Science Repts. Tôhoku Univ., First Series*, **37**, 49 (1953); J. B. Willis and D. P. Mellor, *J. Amer. Chem. Soc.*, **69**, 1237 (1947).

*** Our preliminary investigation by powder method showed that the chelate has probably the tetrahedral configuration.

*A New Analytical Method of Electroconductivity Data of Surfactant Solutions. I**

By Kenjiro MEGURO, Tamotsu KONDO, Noriaki OHBA, Takashi INO and Osamu YODA

(Received December 15, 1956)

In the estimation of *C. M. C.* value by the electroconductivity method, the equivalent conductivity (Λ) *vs.* square root of concentration ($\sqrt{c}$) diagram or the specific conductivity (κ) *vs.* concentration (c) diagram plays an important role. In the former diagram the *C. M. C.* has been defined as an intersecting point of two curves; one is a straight line below *C. M. C.* and the other is a concave curve above it.

In many cases, however, the precise examination of experimental data shows that the plots of conductivity in highly diluted regions are not a straight line[1], but a slight maximum appears[2]. The deviation from a straight line is especially conspicuous when surfactant molecules contain a double long chain alkyl group[3] or highly hydrated counter ions[4]. The similar effects upon the conductivity result from the addition of a solvent[5], such as dioxane or alcohol, to surfactant solutions.

On the other hand, it is well known that the Kohlrausch's equation is not applicable exactly to the conductivity of a simple electrolyte. Therefore, it may be unsuitable to treat the plots of electroconductivity data below *C. M. C.* as a straight line, as if they obeyed the Kohlrausch's equation.

In a simple electrolyte solution, the electroconductivity usually obeys a corrected formula such as Onsager's[6] or Shedlovsky's[7] approximate equation:

Onsager's approx. eq.

$$\Lambda=\Lambda_0-A\sqrt{C}+BC, \qquad (1)$$

Shedlovsky's approx. eq.

* Presented at the 9th Annual Meeting of the Chemical Society of Japan held in Kyoto, April, 1956.

1) P. F. Grieger and C. A. Kraus, *J. Am. Chem. Soc.*, **70**, 3803 (1948).
2) M. E. L. McBain, W. B. Dye and S. A. Johnston, ibid., **61**, 3210 (1939).
3) A. W. Ralston, D.N. Eggenberger and P.L. DuBrow, ibid., **70**, 977 (1948).
4) G. L. Brown, P. F. Grieger and C. A. Kraus, ibid., **71**, 95 (1949).
5) E. C. Evers and C. A. Kraus, ibid., **70**, 3049 (1948).
6) L. Onsager, *Physik. Z.*, **27**, 388 (1926).
7) T. Shedlovsky, *J. Am. Chem. Soc.*, **54**, 1405 (1932).

TABLE I

COMPARISON OF THE CONDUCTIVITY AT THE MINIMUM OR THE MAXIMUM POINT WITH THE *C. M. C.* ESTIMATED BY THE USUAL METHODS

Subst.	C_{min}	C_{max}	*C. M. C.* (in mM./l.) estimated by' Elect. conductivity	Dye	Surface tension	Viscosity	Solubilization	Light scatt.	Transport number	Osmotic coeff.	Vapor press.
$C_{10}SO_3Na$	40° 40	40° 91.5	40° 40	38.7	40° 36.4						
$C_{10}SO_4Na$				room 26.2							
C_{10} K soap				25° 99.8			71.4				
$C_{10}N^+(CH_3)_3Br^-$	40° 51.0	40° 103.7	40° 70	63.6				70			
$C_{12}SO_3Na$	40° 10.5	40° 27.2	40° 11.0	23.5° 9.8	40° 10.0	40° 12	10				
$C_{12}SO_4Na$	30° 6.08	30° 20.2	40° 8.9	room 5.8	40~75° 6~9.6						30° 7.0
C_{12} K soap				25.8° 23.5			21.4			11.1	20.5
C_{12} Na soap	40° 22.5	40° 59	40° 28			96	50° 26				27
$C_{12}N^+(CH_3)_3Br^-$	40° 12.1	40° 34.2	40° 17.5					15			
$C_{12}N^+$⬡Br^-			30° 12.0	room 11.5							30° 20.6
$C_{12}NH_3Cl$	60° 12.1	60° 44.1	60° 13			15	14		30° 15		30° 16.5
$C_{14}SO_3Na$	40° 2.71	40° 8.51	40° 2.5		40° 2.6						
$C_{14}NH_3Cl$	60° 2.91	60° 16.1	60° 4.0						30° 4.0		

$$\Lambda_0 = \frac{\Lambda - \beta\sqrt{C}}{1 - \alpha\sqrt{C}} - BC, \qquad (2)$$

in which A, B, α and β are constants, and BC is a corrected term for the higher order interaction between ions.

The fact of micelle formation of surfactant ions suggests that the interaction of those ions can not be neglected even in an appreciably dilute region below *C. M. C.* Accordingly, it is more reasonable to adopt the Onsager's equation (1) etc. than the Kohlrausch's equation, because we must consider the contribution of the interaction between surfactant ions to the conductivity in solution of the concentration below *C. M. C.* The deviation from the Onsager's equation (1) must be considered as "real deviation from the simple electrolyte".

The order of approximation of Shedlovsky's equation is higher than that of Onsager's equation, but we use the Onsager's equation (1) in this report to simplify the calculation and the analysis.

Now we defined the quantity;

$$(\Lambda_0 - \Lambda)/\sqrt{C}, \qquad (3)$$

which is denoted by H and can be expressed as follows;

$$H = (\Lambda_0 - \Lambda)/\sqrt{C} = A - B\sqrt{C}. \qquad (4)$$

If the quantity H is plotted against $\sqrt{C}$, the H *vs.* $\sqrt{C}$ diagram becomes a straight line in the case of the simple electrolyte, and the intercept on the axis of H is equal to A, while the slope of the plots gives B, the coefficient of corrected term.

In the case of surfactant solution, the deviation, from the linearity is observed in diluted region, that is, the remarkable minimum or kink and maximum are found in the diagram. Therefore, we use merely the value B as a parameter to appraise the slope of curve and not to show the corrected term exactly.

This deviation can be considered as "real deviation from the simple electrolyte", and will be due to the micelle formation. In Fig. 1 the relation between the

usual *C. M. C.* and the concentration at the minimum or the maximum point is shown by the arrow line.

It is noticed here that the value Λ_0 which is necessary to draw the above diagram was obtained by the extrapolation of the plots to infinite dilution in usual Λ *vs.* $\sqrt{C}$ diagram, because the data in very diluted concentration—more diluted than the concentration where the slight maximum appears—can be treated as being in accordance with the Kohlrausch's equation.

The concentration of minimum does not always coincide with the usual *C. M. C.*; rather it is situated in the lower concentration than the latter. The numerical values of the concentration corresponding to the minimum or the maximum in contrast with the usual *C. M. C.* determined by various methods are listed in Table I. From this table it is found that the usual *C. M. C.* values determined by various methods fall into the region between the minimum and the maximum. We adopt this minimum and this maximum as parameters to show the critical phenomenon in surfactant solution, instead of the *usual C. M. C.*, and will show by the actual example that this method is more reasonable than the usual one.

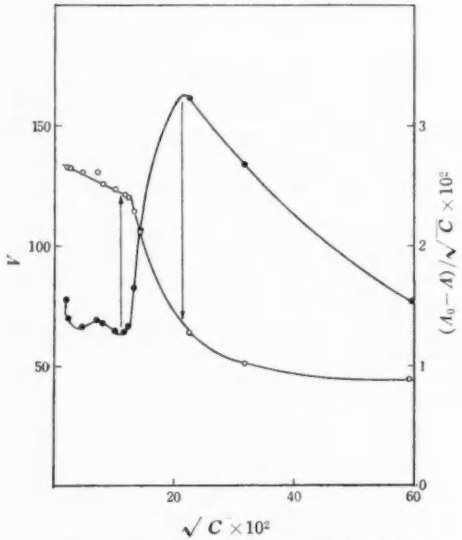

Fig. 1. Comparison of $(\Lambda_0-\Lambda)/\sqrt{C}$ *vs.* $\sqrt{C}$ diagram with the Λ *vs.* $\sqrt{C}$ diagram for dodecyl trimethyl ammonium bromide;
○ Λ *vs.* $\sqrt{C}$, ● $(\Lambda_0-\Lambda)/\sqrt{C}$ *vs.* $\sqrt{C}$.

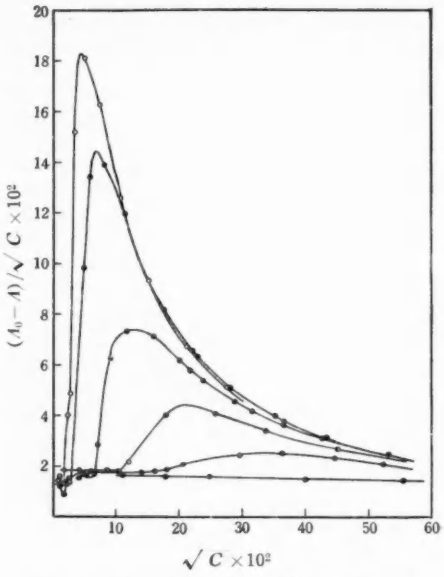

Fig. 2. $(\Lambda_0-\Lambda)\sqrt{C}$ *vs.* $\sqrt{C}$ curves for the aqueous solution of ○ octadecyl-, ● hexadecyl-, ◒ tetradecyl-, ◐ dodecyl-, ◓ decyl-, and ◑ octyl ammonium chlorides at 60°C.

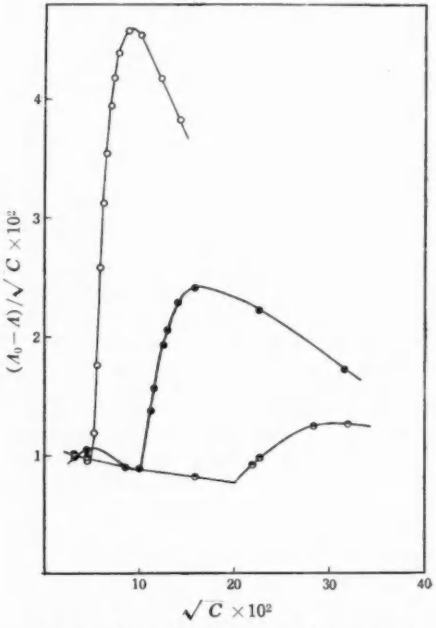

Fig. 3. $(\Lambda_0-\Lambda)/\sqrt{C}$ *vs.* $\sqrt{C}$ curves for the aqueous solution of ○ sodium tetradecyl-, ● sodium dodecyl- and ◓ sodium decyl-sulfonate at 40°C.

Effect of Alkyl Chain Length

The effect of alkyl chain length on the *C. M. C.* has been investigated by some authors and they have concluded that the longer the chain length, the lower the *C. M. C.*

Applying our method to the data of alkyl ammonium chlorides[8,9] and sodium alkyl sulfonates[10], we obtained the following results as shown in Figs. 2 and 3.

(a) Each curve shows a remarkable minimum and a maximum.

(b) The position of the minimum or the maximum shifts toward lower concentration with increasing alkyl chain length.

(c) The value of H at the maximum position increases with the length of alkyl chain.

(d) The slope between the minimum and the maxium becomes steep with the increase in the length of alkyl chain.

Harkins[11] found the following relation between the *C. M. C.* and alkyl chain length.

$$\log C.M.C. = a + bN, \qquad (5)$$

in which a and b are constants and N is the number of carbon atoms in an alkyl radical. A similar relation can be obtained by plotting the logarithm of the position of the minimum or the maximum against the number of carbon atoms in an alkyl radical. Fig. 4 shows the relation between $\log C_{min}$ or $\log C_{max}$ and N.

As the concentration at the minimum is nearly equal to the *C. M. C.* determined by Harkins. it is natural to obtain such a linear relation between the position of minimum and the number of carbon atoms. However, no attention has been paid to the concentration of the maximum position, the magnitude of which is several times that of the usual *C. M. C.* We found that a similar relation also exists between the position of the maximum and the number of carbon atoms. This fact is very interesting and could not be found at all by any of the earlier methods.

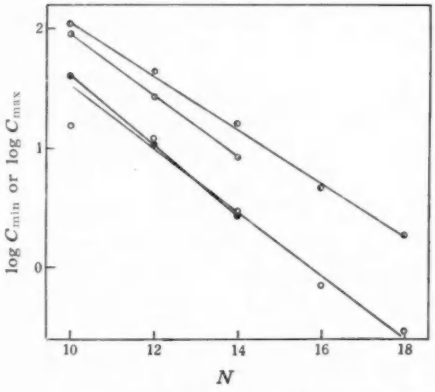

Fig. 4. The relation between logarithm of C_{min} or C_{max} and the number of carbon atoms, N, in an alkyl radical : for alkyl ammonium chlorides, ○ C_{min} and ◐ C_{max}; for sodium sulfonates, ● C_{min} and ◑ C_{max}.

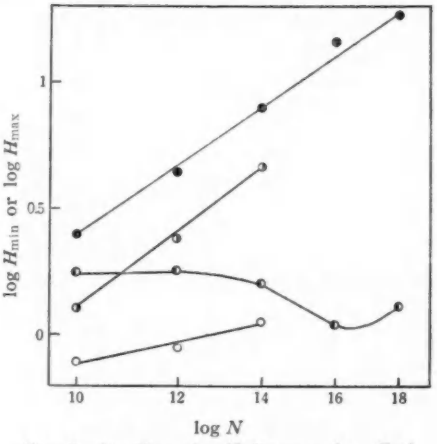

Fig. 5. log H *vs.* log N diagram for alkyl ammonium chlorides, ◐ H_{min}, ● H_{max}; for sodium alkyl sulfonates, ○ H_{min} and ◑ H_{max}.

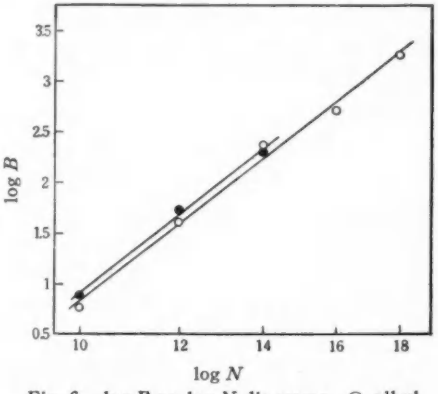

Fig. 6. log B *vs.* log N diagrams, ○ alkyl ammonium chlorides and ● sodium alkyl sulfonates.

8) A. W. Ralston, C. W. Hoerr and K. J. Hoffman, *J. Am. Chem. Soc.*, **64**, 97 (1942).
9) A. W. Ralston and C. W. Hoerr, ibid., **64**, 773 (1942).
10) K. A. Wright, A. D. Abott, V. Sivertz and H. V. Tartar, ibid., **61**, 549 (1939).
11) M. L. Corrin and W. D. Harkins, ibid., **65**, 692 (1943).

On the other hand, when we plot both logarithms of H and B against the logarithm of N, we can also find a linear relation between them. These results are shown in Figs. 5 and 6.

The values of B are almost the same in the case of the samples containing the equal number of carbon atoms. For reference the B values obtained for many examples are shown in Table II. This fact may be used to estimate the alkyl chain length of an unknown surfactant.

TABLE II
B VALUES FOR OBTAINED VARIOUS SURFACTANTS

Substance		B
$C_{18}H_{37}NH_3Cl$	60°	1800
	25°	1530
$N^+(CH_3)_3Br^-$	25°	1200
$C_{16}H_{33}NH_3Cl$	60°	517
		1080
$N^+(CH_3)_3Br^-$	60°	375
$C_{14}H_{29}NH_3Cl$	60°	238
SO_3Na	40°	201
$C_{12}H_{25}NH_3Cl$	60°	41
SO_3Na	40°	56
OSO_3Na	25°	59
$N^+(CH_3)_3Br^-$	60°	35
$C_{10}H_{21}NH_3Cl$	60°	6
SO_3Na	40°	8
$N^+(CH_3)_3Br^-$	60°	6

Effect of the Number of Alkyl Chains

Ralston[12] found an anomalously high Λ value in a low concentration region of the surfactant containing two long alkyl chains, such as dialkyl ammonium salts, and he reported that it was difficult to determine the *C. M. C.* exactly owing to this anomalous maximum.

According to our method, however, it is easy to determine the minimum and the maximum values even in such a case. The result obtained is shown in Fig. 7. The anomality does not appear in our analysis as in the case of compounds with a single long chain, except the fact that the minimum merely becomes more pronounced in this case.

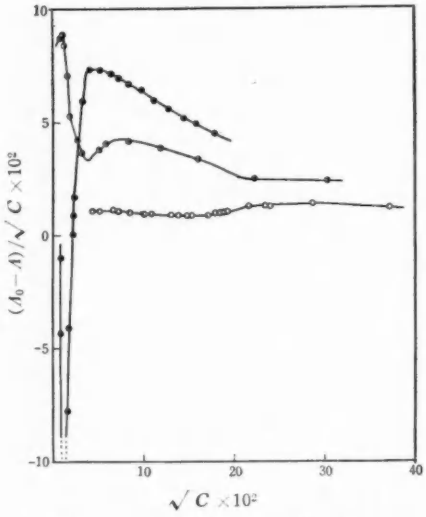

Fig. 7. $(\Lambda_0-\Lambda)/\sqrt{C}$ *vs.* $\sqrt{C}$ diagram, for the aqueous solutions of ○ dioctyl-, ◑ didecyl- and ● didodecyl- dimethyl-ammonium chlorides.

Effect of Ionic Head Group

Now we extend our analysis to the compounds with an alkyl chain of the same length but a different ionic head group.

TABLE III
VALUE OF C_{min}, C_{max}, H_{min}, H_{max}, B AND THE USUAL *C. M. C.*, SHOWING THE EFFECT OF IONIC HEAD GROUP

Substance		C_{min}	C_{max}	H_{min}	H_{max}	B	*C. M. C*
$C_{12}H_{25}SO_3Na$	40°	10.5	27.2	0.89	2.42	56	11.0
$C_{12}H_{25}OSO_3Na$	20°	6.08	20.2	0.50	2.20	58.8	8.6
$C_{11}H_{23}COONa$	40°	22.5	59.0	1.18	1.78	129	28
$C_{18}H_{37}NH_3Cl$	60°	0.78	1.85	4.50	18.3	1800	0.53
$C_{18}H_{37}N^+(CH_3)_3Cl^-$	25°	0.29	0.99	3.65	13.3	1200	0.34
	25°	0.24	1.09	0.30	11.0	1530	0.25

12) A. W. Ralston, D.N. Eggenberger and P.L. DuBrow, *J. Am. Chem. Soc.*, **70**, 977 (1948).

For this purpose the data for sodium dodecyl sulfate[13,14], sodium dodecyl sulfonate[15] and sodium dodecanoate[16] as anionic surfactants, and those for octadecyl ammonium chlorides[8], octadecyl pyridonium chloride[5] and octadecyl trimethyl ammonium chloride[1] as cationic surfactants, were analysed, in which sodium dodecanoate and octadecyl ammonium chloride were chosen especially as hydrolysable surfactants.

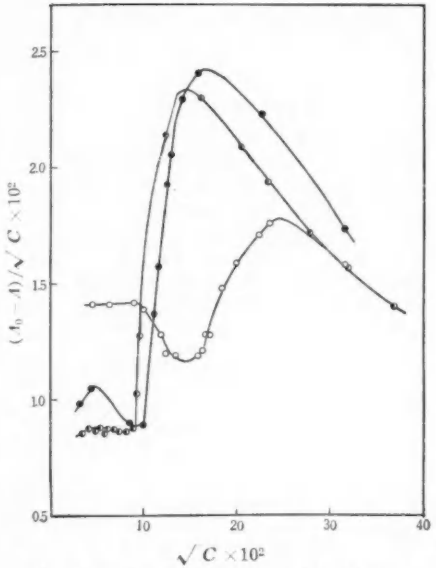

Fig. 8. $(\Lambda_0-\Lambda)/\sqrt{C}$ *vs.* $\sqrt{C}$ diagram for the aqueous solutions of ◐ sodium dodecyl sulfate, ● sodium dodecyl sulfonate and ○ sodium dodecanoate at 40°C.

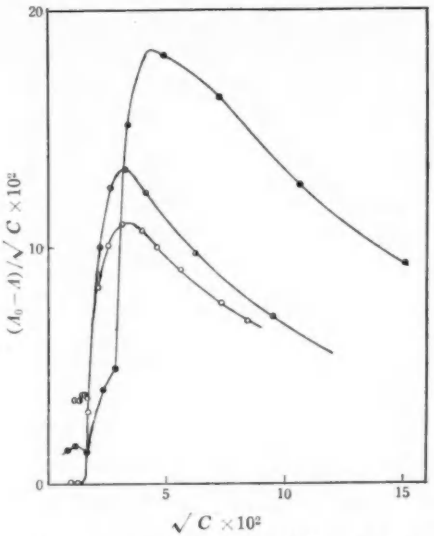

Fig. 9. $(\Lambda_0-\Lambda)/\sqrt{C}$ *vs.* $\sqrt{C}$ diagrams for the aqueous solutions of ● octadecyl ammonium chlorides at 60°C, ◑ octadecyl trimethyl ammonium chloride at 25°C and ○ octadecyl pyridonium chloride at 25°C.

The results are shown in Figs. 8 and 9. The obtained value of C_{min}, C_{max}, H_{min}, H_{max} and B are tabullated in Table III, in which the values of *C. M. C.* estimated by the usual method are given in the last column for reference.

The authors wish to thank Professor B. Tamamushi, Tokyo University, for his encouragement and advice in this work.

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13) A. F. Ward, *Proc. Roy. Soc.*, **A176**, 412 (1940).
14) A. F. Ward, *J. Chem. Soc.*, **1939**, 522.
15) K. A. Wright, A. D. Abott, V. Sivertz and H. V. Tartar, *J. Am. Chem. Soc.*, **61**, 599 (1948).
16) P. Eckwall, *Kolloid Z.*, **101**, 135 (1942).

Oxidimetric and Reductometric Titrations in Neutral Solutions. (Application of Sodium Triphosphate as Masking Agent to Volumetric Analysis.)

By Susumu SUZUKI, Kenji HARIMAYA, Morio UENO, Nobuo TSUJI and Noriko YAMAOKA

(Received December 12, 1956)

In the estimation of metallic ions in neutral media by means of oxidimetric or reductometric titrations, most of them produce insoluble hydroxides or carbonates. In the case of iron (II), its hydroxide is oxidized by the air. So the determination is incomplete. The same is the case with titanium (III). With a view to avoid these effects, the present authors have been trying to apply masking reactions to such cases. In the present paper, they show the use of polyphosphates as the masking agent and as complexing agent, and the extention of the new method will be described. The stability of the reducing standard solutions is also studied.

Oxidimetric Titration

Reagent.—The same oxidizing standard solutions given below were used as in the case of acidic media: one tenth normal solution of ceric sulfate, sodium vanadate, potassium-dichromate, -bromate, -permanganate and -triiodide. Two tenth % solutions of redox indicators were used, namely amarance for bromometry, cacoterin for chromometry, and crystal violet for permanganimetry. Sodium bicarbonate was used for neutralization and sodium triphosphate for masking.

Materials.—One tenth N solutions of iron (II)-, titanium(III)- and chromium(II)- sulfate, sodium -vanadate and -molybdate, thallium(I) nitrate, tin(II)- and antimony(III)- chloride, sodium arsenite and selenium dioxide were prepared (if necessary in an acid medium) and standardized by the usual methods.

Procedure.—Take an aliquot sample solution, add 3g. of solid sodium triphosphate hexahydrate, stir gently, and add solid sodium bicarbonate in small portions until some pieces remain undissolved. Add a few drops of indicator and titrate with the standard solution. The solution should be moderately and constantly stirred in the course of titration. When too much triphosphate is added, a precipitate may appear. If necessary, add more sodium bicarbonate to keep the solution neutral.

Result and Discussion

(*A*) *Permanganimetry.* (Fe^{2+}, Ti^{3+}, VO^{2+}, Cr^{2+}, Mo^{5+}, Tl^{+}.): As the masking force of triphosphate for manganese chloride is incomplete, chlorides should not be present in the sample. In the titration of iron (II), it is completely masked with triphosphate in a neutral medium and the results are satisfactory (Table I). When a fairly

TABLE I

PERMANGANATE METHOD

Element	Sample formula	Element mg.	
		Content	Found
Fe	$FeSO_4$	54.6	54.5
		163.8	163.5
Cr	$CrSO_4$	42.4	42.3
		127.4	127.4
Mo	$Mo_2(SO_4)_5$	55.4	55.4
		166.2	166.7
Ti	$Ti_2(SO_4)_3$	52.1	52.6
		156.3	154.9
Tl	Tl_2SO_4	50.2	50.1
		150.6	149.6
V	$VO(SO_4)$	36.7	36.3
		110.1	109.4

large amont of titanium(III) is titrated, a gelatinous substance is precipitated. The end point is given by the color change from gray to pink. Vanadium(IV) prepared by means of liquid amalgam method, is yellowish green in a neutral solution, and the end point is given by the pink color of permanganate. Chromium(II) is also green in a neutral solution, and it is not difficult to find the end point in permanganimetry. Molybdenum(V) has a pale brown color in a solution containing triphosphate; no precipitate is perceived in solutions of quinquevalent as well as hexavalent molybdenum on addition of triphosphate. Thallium(I) is oxidized to thallium(III), which gives no precipitate (Table I).

(*B*) ***Bromometry*** (Sn^{2+}, Fe^{2+}, Ti^{3+}, As^{3+}, Sb^{3+}, Se^{4+}.): As methyl orange has a yellow color itself in a neutral medium, it is difficult to determine the end point by its decoloration. Amarans is a good redox indicator in a neutral solution which has a red color and is decomposed by oxidizing agents after the element to be oxidised has been quantitatively oxidized. Tin(II) and others are thus completely estimated bromometrically (Table II). The blank for 2 drops of the 0.2% indicator solution is about 0.01 ml. of each 0.1 N oxidizing standard solution.

TABLE II
SODIUM HYPOBROMITE METHOD

Element	Sample formula	Element mg.	
		Content	Found
As	Na_3AsO_3	34.6	34.7
		103.8	104.1
Fe	$FeSO_4$	30.9	31.1
		92.7	92.5
Sb	$SbCl_3$	41.1	41.2
		123.3	123.5
Se	SeO_2	33.8	33.9
		101.4	102.0
Sn	$SnCl_2$	35.2	35.2
		105.6	104.9
Ti	$Ti_2(SO_4)_3$	43.2	43.0
		129.6	128.6

(*C*) ***Chromometry*** (Fe^{2+}, Ti^{3+}, Sn^{2+}, Sb^{3+}.): The leuco compound is formed when methylene blue is added to iron(II) sample solution; at the end point, the former is oxidizied to the colored state. In the case of titanium(III) or tin(II), cacoterin is added which combines with these metals forming colored compounds that are decomposed and decolored at the end point. In the estimation of antimony(III), the end point is determined when the yellow color of chromate is perceived in the green color of the solution (Table III).

TABLE III
POTASSIUM DICHROMATE METHOD

Element	Sample formula	Element mg.	
		Content	Found
Fe	$FeSO_4$	45.3	45.2
		135.9	136.4
Sb	$SbCl_3$	37.6	37.2
		112.8	111.4
Sn	$SnCl_2$	30.9	29.9
		92.7	91.5
Ti	$Ti_2(SO_4)_3$	43.2	43.6
		129.6	127.8

(*D*) ***Vanadometry*** (Fe^{2+}, Ti^{3+}, Sn^{2+}, Sb^{3+}): The metal ions are estimated using methylene blue for iron(II), titanium(III) and molybdenum(V), and cacoterin for tin(II) (Table IV).

In the above-mentioned oxidation titrations, the effect of the following elements was studied:

Li, Na, Ca, K, Sr, Ba, Mg, Cu, Zn, Cd, B, Al, Pb, Nb, As, Mn, Sb, Cr, Ni, Co, Bi, F, Si, Zr, Th, Ce, Tl, Be, W, Ag, Hf, U, Mo, Sn, Fe, Pt, Os, Rh, Pd, and Au.

TABLE IV
SODIUM VANADATE METHOD

Element	Sample formula	Element mg.	
		Content	Found
Fe	$FeSO_4$	45.2	44.8
		136.5	135.1
Mo	$Mo_2(SO_4)_3$	55.4	55.0
		166.2	165.4
Sn	$SnCl_2$	30.9	29.9
		92.7	91.3
Ti	$Ti_2(SO_4)_3$	37.6	37.1
		112.8	110.9

Of these, Ag, Au, Hg, Cu, Sn, Sb, As, and Pd interfere with the determination of iron(II) and titanium(III), Ag, Sn, Sb, Pd and U with that of arsenic(III), and Ag, Hg, Au, Sn, As, Pd and Mo with that of antimony(III).

(*E*) ***Mixture of iron(II) and titanium (III)***: Total concentration of these metals is estimated by direct titration with permanganate solution (Second column in Table V). On the other hand, titanium (III) can be masked with ammonium bifluoride in the permanganate method; iron(II) can thereby be estimated after its addition, methylene blue being used as indicator (First and fourth column in Table V). Titanium(III) is estimated from the difference between these two titres (First and second column).

(*F*) ***Mixture of iron(II) and vanadium (IV)***: Total concentration can be determined by direct titration with permanganate solution without indicator. As the leuco compound of methylene blue is oxidized before vanadyl ion is oxidised, iron (II) can be estimated. From the difference between these two titres, vanadium(IV) can be estimated (Table VI).

(*G*) ***Mixture of titanium(III) and vanadium(IV)***: Total concentration can be determined by direct titration with permanganate solution without indicator, and titanium(III) can be estimated by the use of methylene blue (Table VII).

TABLE V

MIXTURE OF IRON(II) AND TITANIUM(III)

0.1229 N $KMnO_4$ used ml.		Fe mg.		Ti mg.	
NH_4HF_2 & $Na_5P_3O_{10}$ as masking agent & methylene blue as indicator	$Na_5P_3O_{10}$ as masking agent without indicator	Content	Found	Content	Found
3.78	8.08	26.2	25.9	25.6	25.4
3.78	16.64	26.2	25.9	76.1	75.7
7.59	20.46	52.3	52.1	76.1	74.8
11.38	15.66	78.5	78.1	25.4	25.2
11.35	19.97	78.5	77.9	50.7	50.6

TABLE VI

MIXTURE OF IRON(II) AND VANADIUM(IV)

0.1229 N $KMnO_4$ used ml.		Fe mg.		V mg.	
$Na_5P_3O_{10}$ as masking agent & methylene blue as indicator	$Na_5P_3O_{10}$ only	Content	Found	Content	Found
3.77	6.94	25.8	25.8	19.9	19.9
3.75	13.24	25.8	25.7	59.5	59.3
7.49	16.98	51.7	51.4	59.5	59.5
11.27	14.42	77.5	77.4	19.9	19.6
11.24	17.56	77.5	77.3	39.7	39.6

TABLE VII

MIXTURE OF TITANIUM(III) AND VANADIUM(IV)

0.1229 N $KMnO_4$ used ml.		Ti mg.		V mg.	
$Na_5P_3O_{10}$ as masking agent & methylene blue as indicator	$Na_5P_3O_{10}$ only	Content	Found	Content	Found
4.61	7.83	27.0	27.0	20.2	20.1
4.57	14.18	27.0	27.0	60.5	60.2
9.16	18.75	53.9	53.9	60.5	60.1
13.73	17.07	80.9	80.8	20.2	20.1
13.71	20.22	80.9	80.7	40.3	40.1

TABLE VIII

MIXTURE OF CHROMIUM(II) AND TITANIUM(III)

0.0898 N $KMnO_4$ used ml.		Cr mg.		Ti mg.	
NH_4HF_2 & $Na_5P_3O_{10}$ as masking agent & methylene blue as indicator	$Na_5P_3O_{10}$ only	Content	Found	Content	Found
2.71	8.89	12.7	12.6	26.6	26.6
2.69	21.22	12.7	12.6	79.8	79.6
5.41	23.93	25.4	25.3	78.9	79.8
8.12	14.24	38.1	37.9	26.6	26.3
8.11	20.49	38.1	37.9	53.2	53.1

(*H*) *Mixture of chromium(II) and titanium(III)*: Total concentration is estimated by direct titration with permanganate solution without indicator; after masking titanium(III) with ammonium bifluoride, chromium(II) is determined similarly. Titanium(III) can be estimated from the difference between these titres (Table VIII).

(*I*) *Mixture of iron(II), titanium(III) and vanadium(IV)*: Total concentration can be determined by means of direct titration with permanganate solution without indicator (Third column in Table IX). The sum of the concentrations of iron(II) and titanium(III) can be determined by direct titration in the presence of methylene blue (Second column in Table IX).

TABLE IX

MIXTURE OF IRON(II), TITANIUM(III) AND VANADIUM(IV)

0.0998 N $KMnO_4$ used ml.			Fe mg.		Ti mg.		V mg.	
NH_4HF_2 & $Na_5P_3O_{10}$ as masking agents & methylene blue as indicator	$Na_5P_3O_{10}$ only as masking agent & methylene blue as indicator	$Na_5P_3O_{10}$ without indicator	Content	Found	Content	Found	Content	Found
5.09	11.28	15.59	25.7	25.5	26.7	26.6	20.1	20.1
10.21	28.78	33.01	51.3	51.2	80.0	78.9	20·1	19.8
15.38	21.56	25.79	77.0	77.1	26.7	26.5	20.1	19.8
15.35	27.74	40.42	77.0	76.9	53.3	53.3	60.4	59.2
15.36	33.88	38.12	77.0	77.0	80.0	79.6	20.1	18.9
5.09	23.76	36.52	25.7	25.5	80.0	79.8	60.4	59.7

Iron(II) is estimated by means of masking titration, in the presence of ammonium bifluoride and methylene blue (First column in Table IX). From these three titrations, titanium(III) and vanadium(IV) are estimated.

Reductometric Titration

Reagent.—Add 20 g. of sodium triphosphate to one liter of 0.1 N acidic standard solution, cautiously neutralized with solid sodium bicarbonate until no more carbone dioxide is evolved. Stock the solution in a dark box. Iron(II)-, titanium (III)-, vanadium(IV)-, and chromium(II)- triphosphate solution are fairly stable. Indigo carmine, methylene blue, methyl violet, crystal violet, and amarance are used as indicators.

Material.—As the sample, solution of potassium -permanganate, -bichromate, cerium(IV)-, and iron(III)- sulfate were used, their titres being determined beforehand in acidic solutions.

Procedure.—Add 3 g. of sodium triphosphate hexahydrate to an aliquot sample solution, and neutralize it with sodium bicarbonate, avoiding an excess. Titrate the mixture with a standard solution.

Result and Discussion

(*J*) *Ferrousmetry* (MnO_4^-, IO_4^-, Ce^{4+}, CrO_4^{2-}, VO_3^-, BrO^-, Br_2, H_2O_2). Potassium -permanganate and -iodate are titrated with a reducing standard solution directly: the color of the sample solution disappears at the end point. In the cases of cerium(IV) sulfate, potassium chromate, sodium vanadate and hydrogen peroxide, cacoterin is used as indicator which imparts a violet coloration to the medium containing an excess of iron(III) solution. In the determination of hypobromite (or bromine), the indicator is decomposed before the end point, so that it is necessary to reduce BrO^- with an excess of iron(II) solution (Table X).

(*K*) *Chromometry* (IO_3^-, I_3^-, MnO_4^-, CrO_4^{2-}, VO_3^-, Ce^{4+}, Fe^{3+}, BrO^-). The solution of potassium triiodide is decolorized at the end point. In the cases of potassium -permanganate and -chromate, sodium vanadate and cerium(IV) sulfate, indigocarmine is used; neutral red may be used for chromate and neutral red as well as crystal violet for iron(III). Hypobromite is determined by back titration as in ferrousmetry (Table XI).

TABLE X

FERROUSMETRY

Element	Sample formula	Element mg. Content	Found
Br	NaBrO	40.7	40.6
		125.8	125.7
Ce	$Ce(SO_4)_2$	56.2	56.0
		170.8	170.6
Cr	K_2CrO_4	29.3	29.2
		87.7	87.6
	H_2O_2	22.9	22.8
		68.2	68.2
	I_2	KI_3	67.8	67.5
		202.4	201.6
Mn	$KMnO_4$	33.8	33.7
		101.7	101.7
V	$NaVO_3$	23.5	23.5
		71.7	71.5

TABLE XI

CHROMOMETRY

Element	Sample formula	Element mg. Content	Found
Br	NaBrO	37.6	37.2
		112.7	112.2
Ce	$Ce(SO_4)_2$	56.2	55.9
		170.8	170.5
Cr	K_2CrO_4	26.2	26.2
		78.6	78.5
Fe	$Fe_2(SO_4)_3$	10.6	10.3
		59.1	58.8
I	KI_3	67.8	67.5
		202.4	201.6
Mn	$KMnO_4$	33.8	33.7
		101.7	101.7
V	$NaVO_3$	16.1	15.8
		49.1	48.7

(L) Vanadyl sulfate method (MnO_4^-, CrO_4^{2-}, Ce^{4+}). Permanganate is the only ion determinable by direct titration. For chromate and cerium(IV) sulfate, an excess of vanadyl sulfate is added and back-titrated with standard iron(III) sulfate solution (Table XII).

TABLE XII
VANADYL SULFATE METHOD

Element	Sample formula	Element mg.	
		Content	Found
Ce	$Ce(SO_4)_2$	56.2	56.9
		170.8	170.6
Cr	K_2CrO_4	26.2	26.0
		78.6	78.0
Mn	$KMnO_4$	37.3	37.2
		110.9	110.7

(M) Mixture of chromate and iron(III). Chromate is reduced with chromium(II) solution; indigo carmine is decolorized at the end point and used as indicator. Total concentration of chromate and iron (III) is determined by the titration using crystal violet as indicator which is reduced by the standard solution with more difficulty than iron(III); iron is estimated from the difference between these two titrations (Table XIII).

TABLE XIII
MIXTURE OF IRON(III) AND CHROMATE

0.0921 N $CrSO_4$ required ml. $Na_5P_3O_{10}$ as masking agent		Cr mg.		Fe mg.	
Indigo carmine	Crystal violet	Content	Found	Content	Found
5.47	8.94	26.2	26.1	19.6	19.5
5.45	15.96	26.2	26.0	59.2	58.9
10.93	17.86	52.6	52.2	59.1	58.9
16.39	19.01	78.6	78.2	19.6	19.0
16.42	23.35	78.6	78.4	39.4	39.0

(N) Mixture of vanadate and iron(III). Indigo carmine is used as indicator in the chromometric titration of vanadate; the total concentration of these two components is determined by the use of neutral red as indicator. (Table XIV).

TABLE XIV
MIXTURE OF IRON(III) AND VANADATE

0.0921 N $CrSO_4$ solution required $Na_5P_3O_{10}$ as masking agent		V mg.		Fe mg.	
Indigo carmine	Neutral red	Content	Found	Content	Found
2.42	5.21	15.6	15.1	19.6	19.1
2.43	11.04	15.6	15.1	59.1	58.8
4.99	13.59	31.2	31.1	59.1	58.8
7.46	10.25				
7.44	13.14	46.9	46.5	39.4	39.0

Summary

Antimony(III), arsenic(III), chromium (II), iron(II), molybdenum(V), selenium (IV), thallium(I), titanium(III) and vanadium(IV) are titrated in neutral solutions with oxidizing standard solutions, chromate, bromate, hypobromite, permanganate, and vanadate solution, in the presence of triphosphate. A few of these elements, chromium(II), iron(II), titanium(III) and vanadium(IV) in their mixture are also determined.

On the other hand, cerium(IV), hypobromite, chromate, triiodide, permanganate, vanadate and hydrogen peroxide, are titrated with reducing standard solutions, chromium(II), iron(II), titanium(III), and vanadyl solution containing triphosphate. Some of them in mixtures are also determined.

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Determination of Sulfate by Means of Metal-Polyphosphate Reduction Method

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Various methods for the estimation of sulfate by reduction to hydrogen sulfide have been known, and recently Madsen[1], Johnson[2], Kiba[3], and Ohashi[4] have given procedures for volumetric determination of sulfate. Madsen used a mixture of tin (II) chloride and phosphoric acid as reductant. But his method is applicable only to the determination of sodium sulfate. Johnson and Nishita[2] have shown methods for micro determination of sulfur, sulfate and organic sulfur compounds using a reducing mixture of hydroiodic acid and red phosphorus at 115°C. Kiba gives a new procedure involving the use of tin(II)-strong phosphoric acid mixture. By his method, sulfur in barium, magnesium, sodium and zinc sulfate as well as aluminium and chromium alum, are estimated. Ohashi reported also on the rapid determination of sulfur in organic compounds by means of the tin(II)-strong phosphoric acid method. According to his method, nitro compounds are determined within 0.5% accuracy. On the other hand, a few applications of triphosphate have been reported by the present authors. In the present paper, will be described the determination of soluble and insoluble sulfate by means of new reducing agents, metal-polyphosphate systems: titanium(III), chromium(II), molybdenum(IV), tungsten (V), and vanadium(IV), pyrophosphate, triphosphate and mixed polyphosphate systems.

Equipment and Material

Equipment.—The apparatus for reduction of sulfate consists of two parts, (A) reduction- and (B) absorption- vessel. For the former a 50 ml. pear-shaped flask is used in a reaction below 120°C or a 10 ml. porcelain boat between 700°C and 900°C. The flask is provided with one glass tube for introduction of carbon dioxide and another one, through which the resultant gas mixture can be drawn to the absorption vessels. The boat is inserted into a silica tube, (length, ca. 40 cm. diameter, ca. 2 cm.) which is provided also with gas inlet- and delivery- tube as mentioned above. For the latter three 150 ml. flasks connected to each other with ruber tubings are used; the first is a gaurd flask and the remaining two are for absorption. A porcelain crucible No.5 is used for fusion of the mixture of polyphosphate and powdered metal.

Material.—Sodium sulfite, and barium, lead, and sodium sulfate were used as samples. The purity of lead and sodium sulfate was determined gravimetrically, while sodium sulfite was estimated iodimetrically. Barium sulfate was prepared from barium chloride and potassium sulfate.

Standard Solution.—One tenth normal potassium triiodide solution was used as absorbent, and sodium thiosulfate solution of the same normality as a reference standard solution. No indicator was used in the titration.

Titanium Polyphosphate Systems. — (a) Five g. of metallic titaninm is added to 200 g. of orthophosphoric acid (sp. gr. 1.86), which is boiled to dissolve the metals. Titanium is oxidized to tervalent state; even if colorless quadrivalent ion should be formed here, it would be reduced to tervalent state by the elementary one. Tervalent titanium in polyphosphate is very stable in the cold and has a vigorous reducing force at high temperatures. In separate experiments, 0.3 g. portions of titanium are dissolved in mixtures containing each 5 g. of orthophosphoric acid, (sp. gr. 1.83) along with (b) 2.5 g. of pyrophosphate, (c) 2.5 g. of triphosphate, (d) 2.5 g. of hexametaphosphate, and (e) 5g. of a mixture of the hexameta- and tri- phosphate in equal proportion respectively; (a) and (b) are used at 200°C, while (c), (d) and (e) at 700°—900°C.

Chromium-, Manganese-, Molybdenum-, and Tungsten- Polyphosphate Systems.—Metallic chromium is dissolved in phosphoric acid; the solution which exerts a reducing force at a high temperature, contains the metal in bivalent and tervalent state and is of green color. Chromium-polyphosphate reducing systems as well as those containing manganese, molybdenum and tungsten are prepared as in the case of titanium.

Vitreous Polyphosphates. — Mixtures prepared from phosphoric acid and pyro-, tri- and hexa- phosphate without the additon of metals are used as diluents.

1) E. R. Madsen, *Acta. Chim. Scand.*, **3**, 773 (1949); **6**, 305 (1952).
2) C. M. Johnson and H. Nishita, *Anal. Chem.*, **24**, 736 (1952).
3) T. Kiba, This Bulletin, **28**, 641 (1955).
4) S. Ohashi, This Bulletin, **28**, 645 (1955).

Procedure

Place a sample of approximately 0.01 g. of air dry sulfate in the reduction flask or in the ignition boat. Add ca. 7 g. of reducing metal-polyphosphate mixture. To each absorber for hydrogen sulfide, add 25 ml. of one tenth normal potassium triiodide solution and 20 ml. of water. Pass a current of carbone dioxide for at least 2 minutes through the reduction flask or the silica tube into which the ignition boat is inserted, in order to remove atmospheric oxygen. Allow the reaction to proceed gradually at a boiling temperature of the mixture in the current of carbon dioxide for about 20 minutes. After heating, the potassium triiodide solution, in which hydrogen sulfide gas is absorbed, is titrated with standard sodium thiosulfate solution. One ml. of one tenth normal solution of sodium thiosulfate corresponds to 7.103 mg. of sodium sulfate, 6.9043 mg. of sodium sulfite, 14.377 mg. of zinc sulfate monohydrate, 15.164 mg. of lead sulfate and 11.671 mg. of barium sulfate.

Result and Discussion

Titanium Polyphosphate Systems.—(1) When barium, lead, or sodium sulfate is treated with H_3PO_4–Ti reducing system at about 200°C for 15–20 minutes, it is reduced to sulfide; the evolved hydrogen sulfide is absorped in standard potassium triiodide solution and the excess of triiodide is titrated with standard sodium thiosulfate solution. The results are satisfactory as shown in Table I. Sodium sulfite can not be reduced, however, to sulfide by this reducing system, presumably because the sulfide is easily decomposed by the heat treatment, and the resultant sulfur volatilizes at a high temperature. The results are shown in Table I.

TABLE I
H_3PO_4–Ti SYSTEM

Sample form	Heating time (min.)	Sample mg. Content	Found
K_2SO_4	20	blanck	0.0
	15	12.2	11.8
	〃	30.4	29.9
	〃	61.9	61.1
	25	123.6	101.1
$PbSO_4$	15	33.4	33.9
$BaSO_4$	15	28.6	28.0
	〃	32.3	31.9
	20	61.7	62.3
	30	97.4	81.8
	〃	122.3	95.5
Na_2SO_3	20	29.5	29.4
	30	67.9	62.8

(2) Solid samples of sulfates are reduced also by means of H_3PO_4–$(HPO_3)_6$–Ti system. In this case, the samples are heated at a higher temperature, about 200°C. for 15 minutes. The results are shown in Table II. A small quantity of sodium sulfite is reduced completely, but such is not the case with its large amount.

TABLE II
H_3PO_4–$(HPO_3)_6$–Ti SYSTEM

Sample form	Heating time (min.)	Sample mg. Content	Found
K_2SO_4	15	46.8	46.4
	〃	83.7	83.9
$PbSO_4$	〃	40.1	40.4
$BaSO_4$	〃	27.0	26.9
Na_2SO_3	〃	18.1	17.8

(3) When H_3PO_4–$Na_5P_3O_{10}$–Ti system is used as a reducing agent, sulfates are determined satisfactorily as shown in Table III. Nevertheless it must be recognized that their large amounts can not be completely reduced by this method. This owes to the fact that the reaction temperature is higher than in other cases.

TABLE III
H_3PO_4–$Na_5P_3O_{10}$–Ti SYSTEM

Sample form	Heating time (min.)	Sample mg. Content	Found
K_2SO_4	10	27.4	27.2
	15	57.9	57.5
	〃	91.3	82.1
$PbSO_4$	〃	60.9	61.1
	〃	102.4	94.3
$BaSO_4$	〃	68.5	68.3
	〃	102.0	100.4
Na_2SO_3	10	32.8	32.6
	15	31.0	30.9
	〃	63.0	53.9

TABLE IV
H_3PO_4–$NaPO_3$–Ti SYSTEM

Sample form	Heating time (min.)	Sample mg. Content	Found
H_2SO_4	25	21.1	21.6
K_2SO_4	15	22.0	21.8
$PbSO_4$	〃	24.6	24.4
$BaSO_4$	〃	23.0	23.2
Na_2SO_3	〃	9.5	9.6

(4) The same fact is also observed in H_3PO_4–$NaPO_3$–Ti reducing system. About 20 mg. of sulfate are treated with the reductant in a pear-shaped flask for about

15 minutes, and found to be reduced completely, but a sample weighing more than 20mg. can not be determined satisfactorily. The results are shown in Table IV.

(5) A reducing agent prepared from H_3PO_4, HPO_3, $Na_5P_3O_{10}$ and metallic titanium is used at about 700°C. The reaction velocity being still small, the result after 20 minutes' heating is unsatisfactory; one hour is necessary for complete reduction. The results are shown in Table V.

TABLE V

H_3PO_4-$(HPO_3)_6$-$Na_5P_3O_{10}$-Ti SYSTEM

Sample form	Heating time (min.)	Sample mg. Content	Found
K_2SO_4	20	32.3	29.3
	60	36.5	36.7
$PbSO_4$	20	35.8	30.4
	60	57.8	57.8
$BaSO_4$	20	34.1	29.7
	60	45.9	45.7
Na_2SO_3	20	34.5	25.1
	40	30.0	25.1
	60	38.4	33.0

(6) Table VI shows the results obtained from reactions between H_3PO_4-$Na_4P_2O_7$-Ti reducing agent and sulfates. The reacting mixture is heated in the boat at 800°C; one hour's heating is necessary.

TABLE VI

H_3PO_4-$Na_4P_2O_7$-Ti SYSTEM

Sample form	Heating time (min.)	Sample mg. Content	Found
K_2SO_4	30	22.0	16.4
	60	29.0	28.7
$PbSO_4$	30	32.1	32.6
	60	37.4	37.6
$BaSO_4$	30	42.1	37.6
	60	41.9	41.7
Na_2SO_3	30	21.3	16.4
	60	20.4	17.8

TABLE VII

H_3PO_4-$(HPO_3)_6$-$KNaCO_3$-Ti SYSTEM

Sample form	Heating time (min.)	Sample mg. Content	Found
K_2SO_4	30	16.6	11.1
	60	12.3	12.2
$PbSO_4$	30	17.9	12.2
	60	13.5	13.6

(7) When potassium-sodium carbonate is added to the reducing system, its melting point rises, which makes the treatment of reducing reaction more difficult. When heated for 60 minutes however, sulfates are satisfactorily determined as shown in Table VII.

Other Metal-Polyphosphate Systems.—(8) H_3PO_4-$Na_5P_3O_{10}$-metal systems. Metallic chromium, manganese, molybdenum or tungsten are dissolved by heating in a mixture of phosphoric acid and sodium triphosphate, and used as reducing agent. But the results of the estimation are satisfactory only in the case of the chromium system as shown in Table VIII.

TABLE VIII

H_3PO_4-$Na_4P_2O_7$-Cr SYSTEM

Sample form	Heating time (min.)	Sample mg. Content	Found
K_2SO_4	15	34.2	34.2
$PbSO_4$	〃	35.7	35.4
$BaSO_4$	〃	30.7	30.7

(9) In order to increase acidic properties and rise the melting point, metaphosphate is added to this system; the sulfate is completely reduced by the reductant as shown in Table IX. These estimations required a heating period of 1 hour.

TABLE IX

H_3PO_4-$(HPO_3)_6$-Na_3PO_4-METAL SYSTEMS

Metal	Sample form	Heating time (min.)	Sample mg. Content	Found
Cr	K_2SO_4	30	11.7	10.1
		60	10.8	10.9
	$PbSO_4$	30	10.7	10.2
		60	11.9	11.8
	$BaSO_4$	30	10.0	7.8
		60	10.4	10.4
Mn	K_2SO_4	30	12.3	11.3
		60	13.4	13.5
	$PbSO_4$	30	17.0	16.2
		60	23.4	23.3
	$BaSO_4$	30	10.7	9.7
		60	16.5	16.5
Mo	K_2SO_4	30	15.6	13.1
		60	20.1	20.1
	$PbSO_4$	30	20.2	19.8
		60	19.2	19.2
	$BaSO_4$	30	15.8	13.3
		60	17.8	18.0
W	K_2SO_4	30	11.6	11.0
		60	11.7	11.8
	$PbSO_4$	30	20.0	16.5
		60	10.0	10.0
	$BaSO_4$	30	10.0	7.7
		60	10.0	10.0

(10) Prolonged heating is also required in the HPO_3–H_3PO_4–$KNaCO_3$–metal system. Sulfates are completely reduced to sulfide by heating for 1 hour with these reducing agents, and the results are shown in Table X.

TABLE X

H_3PO_4–$(HPO_3)_6$–$KNaCO_3$–METALS SYSTEMS

Metal	Sample form	Heating time (min.)	Sample mg. Content	Found
Cr	K_2SO_4	30	12.4	11.5
		60	11.0	11.0
	$PbSO_4$	30	13.7	11.3
		60	10.7	10.7
	$BaSO_4$	30	13.5	12.6
		60	14.3	14.3
Mn	K_2SO_4	30	23.9	21.1
		60	18.8	18.4
	$PbSO_4$	30	21.2	18.8
		60	20.7	20.7
	$BaSO_4$	30	21.7	14.0
		60	15.7	15.7
Mo	K_2SO_4	30	10.6	9.1
		60	10.0	9.9
	$PbSO_4$	30	10.7	9.0
		60	13.8	13.8
	$BaSO_4$	30	13.0	12.5
		60	11.3	11.3
W	K_2SO_4	30	13.2	9.9
		60	10.0	9.9
	$PbSO_4$	30	13.2	12.4
		60	12.2	12.1
	$BaSO_4$	30	10.0	7.5
		60	11.7	11.6

(11) H_3PO_4–$Na_5P_3O_{10}$–metal systems are also used as reducing agents for sulfates, as shown in Table XI. In these systems, chromium is assumed to be present mainly as bivalent, molybdenum as tervalent, manganese as bivalent, and tungsten as quadrivalent compounds respectively, to which the reducing property of the systems is due.

TABLE XI

H_3PO_4–$Na_5P_3O_{10}$–METALS SYSTEMS

Metal	Sample form	Heating time (min.)	Sample mg. Content	Found
Cr	K_2SO_4	30	14.3	12.7
		60	10.4	10.4
	$PbSO_4$	30	9.8	8.3
		60	10.1	10.2
	$BaSO_4$	30	10.4	8.9
		60	10.0	10.0
Mo	K_2SO_4	30	15.9	14.0
		60	16.0	16.0
	$PbSO_4$	30	14.6	13.6
		60	10.6	10.6
	$BaSO_4$	30	10.8	9.1
		60	15.8	15.8
W	K_2SO_4	30	10.0	8.3
		60	11.8	11.8
	$PbSO_4$	30	15.9	13.3
		60	11.0	10.9
	$BaSO_4$	30	9.6	8.1
		60	11.0	10.9

The authours wish to express their hearty thanks to Professor Matsusuke Kobayashi for his valuable advice and encouragement throughout this study.

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Determination of Nitro Compounds with Titanium(III)-Polyphosphate Solution. (Application of Polyphosphate to Masking Volumetric Analysis)

By Susumu SUZUKI, Yoshihiro MURAMOTO, Morio UENO and Teruzo SUGANO

(Received December 12, 1956)

The quantitative reduction of nitro compounds by means of standard titanium(III) chloride solution was proposed by Knecht and Hibbert[1] and applied to many compounds by Duin[2], English[3] and Callen[4]. According to their methods, the sample is dissolved in water or alcohol and excess titanium(III) chloride is added. After the reaction mixture has been boiled, the excess of titanium(III) chloride is titrated with standard iron(III) chloride solution, ammonium thiocyanate being used as indicator. Six equivalents of titanium(III) chloride are required for each nitro group; the nitro nitrogen present in the compound is thereby estimated. On the other hand, new quantitative reduction of sulfate by means of metal-polyphosphate system was reported by S. Suzuki[5]. In the present paper we shall describe the determination of mono-, di-, and tri- nitro compounds by reduction with an excess of standard titanium(III)-polyphosphate solution and back-titration with potassium dichromate, cerium(IV) sulfate, or sodium vanadate solution; indicators which were used are methylene blue, diphenylamine and indigocarmine.

Experimental

Equipment.—A sample of nitro compound is dissolved in water or ethanol in a 200-ml. flask, which is warmed under reflux in a current of carbon dioxide after addition of the standard titanium(III) triphosphate solution.

Material.—Mononitro compounds in Table I, dinitro compounds in Table II, and trinitro compounds in Table III, are used as samples for nitro compounds. These nitro compounds are prepared in the authors' laboratory.

TABLE I
MONONITRO COMPOUND

α-nitronaphthalene
o-nitrophenol
p-nitrotoluene
o-nitroaniline
o-chloronitrobenzene
p-chloronitrobenzene
p-bromonitrobenzene
o-nitrobenzenesulfoamide
4-nitrosalisylic acid
2-nitro-*p*-toluidine
4-nitro-*o*-toluidine
m-nitro-*p*-toluidine
2-acetoamino-4-nitro-toluene
2-nitro-4-acetoamino-toluene

TABLE II
DINITRO COMPOUND

m-dinitrobenzene
2, 4-dinitrotoluene
2, 4-dinitrochlorobenzene
2, 6-dinitro-4-toluidine
di-*o*-nitrophenyldisulfide

TABLE III
TRINITRO COMPOUND

2, 4, 6-trinitrophenol (picric acid)
2, 4, 6-trinitrotoluene

Reagents.—Titanium phosphate solution: 5 g. of metallic titanium is dissolved in 100 ml. of concentrated phosphoric acid (sp. gr. 1.82) by heating at 110—150°C. for about 1 hour. In this case, metallic titanium is gradually added within 10 minutes to the hot phosphoric acid, and stirred carefully not to cause overflow through foaming. This titanium(III) phosphate solution is added to 1 liter of about one tenth normal sodium triphosphate solution. The concentration of tervalent titanium is determined by titration with standard potassium permanganate solution.

Reference Standard Solution. — Potassium dichromate: A tenth normal potassium dichlomate solution is prepared.

Cerium(IV) sulfate: Sixty g. of cerium(IV) sulfate is dissolved in 1 liter of 2 N sulfuric acid, and this solution is used after filtration and standardization.

Sodium vanadate: About one tenth normal solution of sodium vanadate is prepared, and standardized by means of liquid amalgam method.

1) E. Knecht and E. Hibbert, *Ber.*, **36**, 1549 (1903); **38**, 3318 (1905); **40**, 3819 (1907).
2) C. F. van Durin, *Chem. Weekblad*, **16**, 1111 (1919); *Chem. Abst.*, **13**, 3113 (1919).
3) F. L. English, *Anal. Chem.*, **20**, 745 (1948).
4) T. Callan et al., *J. Soc. Chem. Ind.*, **39**, 86 (1920); **41**, 157 (1922).
5) S. Suzuki, This Bulletin, **30**, 766 (1957).

Indicator: 0.1% methylene blue, 0.1% indigo carmine and 0.2% diphenylamine solutions are used as indicators.

Recommended Procedure

Place each sample of accurately measured 0.0300 g. of air dry nitro compounds in a 200-ml. three necksed flask, add 5 ml. of ethanol to dissolve it, and then 25 ml. of a standard titanium polyphosphate solution. Connect a reflux condenser with the flask and a tube into which carbon dioxide has been introduced. Boil the solution well for 5 minutes on a hot plate. Cool the flask in ice water and titrate the solution with a standard oxidizing solution, in a current of carbon dioxide. The indicator is added near the end point, where methylene blue changes color from colorless to blue, indigo carmine also from colorless to blue, while diphenylamine from chocolate violet to pink.

Results and Discussion

Mononitro Compounds: *α-Nitronaphthalene* prepared within 80% yield by means of nitration of naphthalene and recrystallization from ethyl alcohol is estimated with titanium(III) polyphosphate as shown in Table IV. When the sample solution is titrated using methylene blue as indicator, the end point is given a little earlier than the theoretical end point. This is due to the fact that colorless leuco compound of methylene blue produced by reduction of the indicator with titanium (III) salt, is oxidized by standard oxidizing solution before complete oxidation of titanium(III) in solution, which should be oxidized in advance, is achieved. Accordingly it appears as if excess of titanium is consumed by the sample solution and the result shows a higher value. In order to avoid this effect of indicator, iron(II) sulfate is added to the sample solution, in the presence of which methylene blue is not affected till all titanium remaining there has been completely oxidized with the standard solution; then the results show real values as given in the iron(II) sulfate column of Table IV. These facts will also be described hereafter. The result of the estimation of nitronaphthalene by this method is unsatisfactory, the experimental value being 75% of the theoretical one. When indigo carmine is used as indicator, it is oxidized simultaneously with titanium, because of its lower oxidation potential than methylene blue. The end point is determined at the point of decoloration of indigo carmine which is oxidized to colorless leuco compound. In the titration of the solution to which the standard iron(II) sulfate is added, excess of titanium(III) and iron(II) as well as the indicator are all oxidized at the end point. Here lies the difference between methylene blue and indigo carmine. In the presence of triphosphate, in nearly neutral solution, the dichromate ion is changed to the chlomate, whose oxidizing force becomes so weak that it can not oxidize diphenylamine indicator completely; consequently the end point is indistinct. *o-Nitrophenol* is prepared by means of nitration of phenol and separation from *p*-nitrophenol by steam distillation. This nitro compound is determined satisfactorily as shown in Table IV. After nitration of toluene in sulfuric acid medium, *p-nitrotoluene* is separated by means of vacuum distillation and recrystallized from ethyl alcohol; it melts at 56°C. The result of the estimation of *p*-nitrotoluene by the present reduction method corresponds to 72% of nitro group present in the sample. Addition of iron solution to the sample is unsuitable for this compound, except in the case of methylene blue indicator. *o-Nitroaniline* is obtained by nitration of acetanilide in acetic acid anhydride. When methylene blue is used as indicator and iron(II) sulfate is added to the sample solution of *o*-nitroaniline, the titre of cerium(IV) sulfate or sodium vanadate enormously increases, and the estimated value of nitro group correspondingly falls away. A similar fact is also recognized in the determination of 2-nitro-*p*-toluidine described below; it appears as if the resultant diamine compound comes to interfere with the titration. *p-Chloronitrobenzene* is prepared by means of nitration of chlorobenzene in sulfuric acid and recrystallization from ethyl alcohol. The result of determination of this compound is accurate when a sample solution is titrated in the presence of iron(II) sulfate and methylene blue indicator. In the determination of *p-bromonitrobenzene*, diphenylamine shows a pseduo-end point giving rise to a higher result. This compound is prepared by means of nitration of bromobenzene in sulfuric acid, and recrystallization from ethyl alcohol. *o*-Nitrobenzenesulfonylchloride is obtained by oxidation of di-*o*-nitrodiphenylsulfide with chlorine in a mixture of nitric and hydrochloric acid. By means of ammoniation of sulfochloride radical to sulfoamid radical, *o-nitrobenzenesulfoamide* is prepared from *o*-nitrobenzenesulfonylchloride. The determination of this

TABLE IV

DETERMINATION OF MONONITRO COMPOUNDS

Sample	Indicator	Oxidizing agent	Estimated % H_3PO_4—$Na_5P_3O_{10}$	Estimated % $FeSO_4$ added
α-nitronaphthalene	M. B.	Cr	83.01	74.40
		Ce	86.58	65.01
		V	84.34	77.01
	I. C.	Cr	82.34	88.42
		Ce	83.46	78.42
	D. A.	Cr	94.32	excess
		Ce	85.56	76.34
o-nitrophenol	M. B.	Cr	105.82	103.21
		Ce	107.23	101.34
		V	99.68	99.25
	D. A.	Cr	106.35	102.54
		Ce	106.46	97.34
		V	105.99	98.87
p-nitrotoluene	M. B.	Cr	67.44	71.70
		Ce	84.69	67.31
		V	88.64	72.45
	I. C.	Cr	71.32	excess
		Ce	72.32	72.48
		V	72.85	71.63
	D. A.	Cr	70.11	excess
		Ce	73.93	73.34
		V	83.58	73.34
o-nitroaniline	M. B.	Cr	102.23	99.54
		Ce	103.56	trace
		V	101.36	〃
	D. A.	Cr	101.73	excess
		Ce	100.53	99.32
		V	89.79	97.35
p-chloronitrobenzene	M. B.	Cr	100.96	97.35
		Ce	102.35	99.23
		V	102.52	98.32
	I. C.	Ce	97.38	99.01
	D. A.	Ce	excess	101.56
p-bromonitrobenzene	M. B.	Cr	113.34	105.31
		Ce	111.86	102.33
		V	112.81	104.01
	D. A.	Cr	excess	excess
		V	〃	〃
o-nitrobenzenesulfoamide	M. B.	Cr	109.56	104.61
		Ce	105.15	107.07
		V	excess	105.34
	I. C.	Ce	106.36	105.84
	D. A.	Cr	105.41	excess
4-nitrosalisylic acid	M. B.	Cr	114.16	〃
		Ce	100.21	trace
		V	103.56	〃
	I. C.	Ce	101.43	101.32
	D. A.	Ce	101.02	98.72
2-nitro-p-toluidine	M. B.	Ce	93.21	trace
		V	94.62	95.55
	I. C.	Ce	96.30	excess
	D. A.	Cr	excess	〃
		Ce	97.89	95.64
2-acetoamino-4-nitrotoluene	M. B.	Ce	100.85	96.28
		V	102.13	96.51
	I. C.	Ce	113.54	95.86
	D. A.	Ce	96.46	97.37
4-acetoamino-2-nitrotoluene	M. B.	Cr	112.45	98.43
		Ce	108.23	96.58
		V	90.57	96.65
	D. A.	V	90.57	97.78
o-chloronitrobenzene	M. B.	Cr	98.66	trace
		Ce	112.45	〃
		V	98.21	〃
	I. C.	Ce	97.89	97.60
	D. A.	Ce	98.44	97.53
		V	83.05	99.32

TABLE V

DETERMINATION OF DINITRO COMPOUNDS

Sample	Indicator	Oxidizing agent	Estimated % H_3PO_4—$Na_5P_3O_{10}$	Estimated % $FeSO_4$ added
di-o-nitrophenyldisulfide	M. B.	Cr	32.56	28.41
		Ce	54.29	36.78
		V	48.62	30.41
	D. A.	Ce	46.31	trace
2,4-dinitrochlorobenzene	M. B.	Cr	102.56	100.76
		Ce	114.63	trace
	I. C.	Ce	103.52	102.43
	D. A.	Ce	101.01	102.08
		V	103.73	99.60
2,6-dinitro-4-toluidine	M. B.	Cr	excess	98.31
		Ce	97.46	96.53
		V	97.34	95.53
	I. C.	Ce	excess	96.58
	D. A.	Ce	98.13	excess
m-dinitrobenzene	M. B.	Cr	115.38	98.73
		Ce	104.59	98.82
		V	101.10	97.30
	D. A.	Cr	104.12	excess
		Ce	98.02	96.13
		V	99.02	98.34
2,4-dinitrotoluene	M. B.	Cr	101.34	99.47
		Ce	111.22	trace
		V	114.32	〃
	I. C.	Ce	106.35	103.83
		V	99.05	99.43
	D. A.	Ce	100.87	100.51

nitroamide gives a higher result in the similar titration. *4-Nitro-2-aminobenzoic acid* is diazoated at a low temperature, and hydrolyzed by heating the solution to 4-nitrosalisylic acid; the nitrosalisylic acid is recrystallized from the mixture of equal parts of ethyl alcohol and acetic acid. The determination of this compound, using methylene blue as indicator in the presence of iron(II) sulfate, gives a lower result. *p*-Toluidine is nitrated to *2-nitro-p-toluidine* in an excess of sulfuric acid. On the other hand, 2-nitro-*p*-toluidine is also prepared through reduction of dinitrotoluene. The titration of the solution using methylene blue and iron(II) sulfate also gives a lower result. 2-Nitro-4-aminotoluene is acetylated to *2-nitro-4-acetoamino-toluene* and 4-nitro-*o*-toluidine to 2-acetoamino-4-nitrotoluene in acetic acid anhydride, and these acetoaminoes are estimated satisfactorily with iron(II) sulfate-titanium(III)-polyphosphate system.

Dinitro Compounds.—*m-Dinitrobenzene* is prepared by nitration of nitrobenzene and determined as shown in Table V. Although English pointed out that the estimation of dinitro compound gave a higher result, no such discrepancy is observed in the present investigation. *p*-Nitrotoluene is oxidized to 2,4-*dinitrotoluene* by nitration in a large amount of sulfuric acid, and it is purified by recrystallization from ethanolic solution. The dinitro compound is determined by the above-mentioned procedure, but methylene blue-iron(II) sulfate system gives lower results for it. 2,4-*Dinitrochlorobenzene* prepared by nitration of *p*-nitrochlorobenzene, is estimated as in Table V and gives a slightly higher value. When treated with ammonium sulfide in an ethanolic solution, trinitrotoluene, TNT, is subject to partial reduction in *p*-nitro position and changes to *2,6-dinitro-4-toluidine*. This amino compound is satisfactorily determined as shown in Table V. When *o*-chloronitrobenzene is boiled with sodium sulfide in an ethanolic solution, it begins to change into *di-o-nitrophenyldisulfide*. The derivatives can not be estimated by means of the metal-polyphosphate reduction systems.

Trinitro Compound.—*Commercial picric acid* can not be determined in this way, because its solution containing titanium (III) polyphosphate is of a greenish brown color, and so it is impossible to observed the end point. *Trinitro toluene*, TNT, prepared through nitration of dinitrotoluene is determined; the obtained values, though in good agreement with one another, are somewhat lower corresponding to only 96% of theory (Table VI).

TABLE VI
DETERMINATION OF TRINITRO COMPOUNDS

Sample	Indicator	Oxidizing agent	Estimated %	
			H_3PO_4—$Na_5P_3O_{10}$	$FeSO_4$ added
picric acid	M. B.	Cr	96.34	86.42
	I. C.	Ce	107.48	102.45
	D. A.	V	101.35	98.54
2,4,6-trinitro-toluene	M. B.	Cr	excess	98.31
		Ce	97.46	96.53
		V	97.34	95.23
	I. C.	Cr	excess	96.58
	D. A.	Cr	98.13	excess
		V	excess	〃

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Proton Resonance in Oil Paraffin

By Shizuo FUJIWARA and Ichiro YAMAGUCHI

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Introduction

Wilson and Pake[1] proposed a nuclear magnetic resonance method to determine the degree of crystallinity of linear polymers and made an extensive study of the resonance line shape and the spin lattice-relaxation time of polytetrafluoroethylene, 'Teflon.'[2] Though their method is quite straightforward and seems useful for the structural study of linear polymers, further investigations of this method seem necessary, and the present authors have planned to make proton resonance study of oil paraffin which is one of the most typical linear polymers with simple molecular structure and structurally well studied[3].

Experimental

Samples. — Samples investigated are commercial oil paraffins and microcrystalline paraffin waxes. The samples of the former are seven species, from No. 1 to No. 7, differing in their melting points by about 5°F from one another in the range from 115°F (47.7°C) to 145°F (62.8°C); samples from No. 1 to No. 4 are 1 : 1 mixtures of two kinds of crude oil paraffin produced in Ceria and Southern Sumatra, and those from No. 5 to No. 7 are the ones produced in Southern Sumatra.

Apparatus. — The apparatus used for the proton resonance measurement is one of the autodyne—detector type with a narrow band amplifier and phase sensitive detector. The schematic diagram and some descriptions of the apparatus were given elsewhere.[4] The magnetic field applied is of about 4,000 gauss produced by an electromagnet which is charged by storage batteries. The inhomogeneity of the magnetic field over the specimen is about 50 milligauss. Derivative curves are recoreded automatically by the field modulation, whose amplitude is about 0.3 gauss.

Measurement.—For the measurements at room temperature, the oil paraffin specimens are made into rods of 5 mmϕ×15 mm and mounted in a *rf* coil whose axis is perpendicular to the magnetic field. The microcrystalline waxes are sealed in glass tubes with diameters of 5 mm. For the measurements of temperature dependence of the resonance, samples sealed in glass tubes of 3 mm. diameter are mounted in *rf* coil, inserted in a Dewar vessel, and warmed up by a heater. To get the temperature equilibrium in the vessel, about half an hour was taken before the onset of the measurement at each temperature. The temperature range investigated is from room temperature to just below the melting point of the sample. Temperature measurements were made by a copper—constantan thermocouple with one junction 5 mm. apart from the sample.

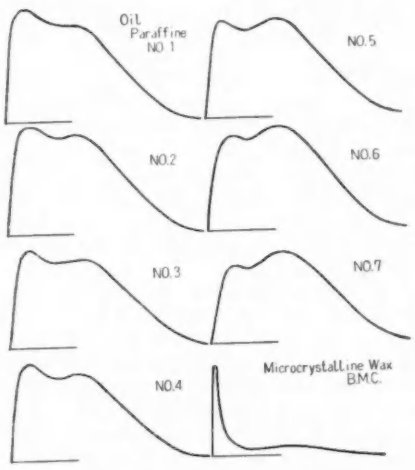

Fig. 1. Experimental derivative curves (at room temperature).

Results and Discussion

Fig. 1 shows the derivative curves of the proton resonance absorption lines at room temperature. According to Wilson and Pake, a resonance curve with the line shape as shown in Fig. 1 may be assumed as a superposition of two lines;[1] a very narrow and a broad one. With the results of their investigations of line shape and relaxation time, they concluded that such a structure of the resonance line was due to coexistence of two components in the sample;[2] one is motional and amorphous and the other rigid and crystalline. According to their proposal, our results may

1) C. W. Wilson III and G. E. Pake, *J. Polymer Sci.*, **10**, 503 (1953).
2) C. W. Wilson III and G. E. Pake, *J. Chem. Phys.* **27**, 115 (1957).
3) A. Müller, *Proc. Roy. Soc.*, **A 120**, 437 (1928); **A 127**, 417 (1930).
4) S. Fujiwara, S. Hayashi and G. Hattori, *J. Chem. Soc. Japan, Ind. Chem. Sec.*, **59**, 803 (1956).

be treated as shown in Fig. 2, where the derivative curve is decomposed into two parts with the assumption that the line

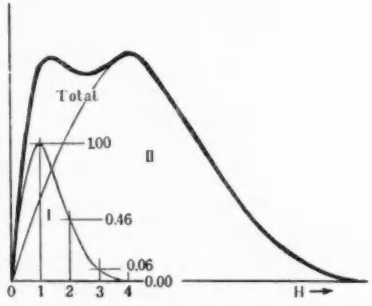

Fig. 2. Decomposition of experimental derivative curve. Experimental curve is decomposed with the assumption that the Part I of the absorption curve is of *Gaussian* type.
—·— Experimental curve
—— Decomposing curve

shape of the narrow part is of Gaussian type. In Fig. 2, two components with narrow line-width and with broad one are referred to I and II, respectively. The line-width, ΔH, defined as the separation between the maxima of the derivative curve, and the second moment, $\langle \Delta H^2 \rangle$, are measured for component II (see Figs. 3 and 4).

The weight percent. of the component II, X_{II}, is also calculated from the area occupied by II in the integrated absorption line (Fig. 5), which is referred to the degree of crystallinity as defined by Wilson and Pake.[1] The correlation time, τ_c or $(1/\nu_c)$, characteristic time scale for the molecular motion which causes the motional narrowing of the line-width, is calculated from the line-width (Fig. 6). In practice, we made a calculation of the correlation time with the assumption that the ΔH value for normal paraffin in rigid state was 15 gauss, being equal to the value for rigid polyethylene.[5]

Since the correlation time, $(1/\nu_c)$, may be related with temperature as $(1/\nu_c)=(1/\nu_0)\exp(V/kT)$,[6] where $(1/\nu_0)$ is a time constant characteristic of the sample, the activation energy V for the molecular motion in the sample to cause the narrowing of the line-width may be calculated by the measurements of the temperature dependence of the line-width.

5) A. Nishioka, private communication.
6) H. S. Gutowsky and L. H. Meyer, *J. Chem. Phys.*, **21**, 2122 (1953).

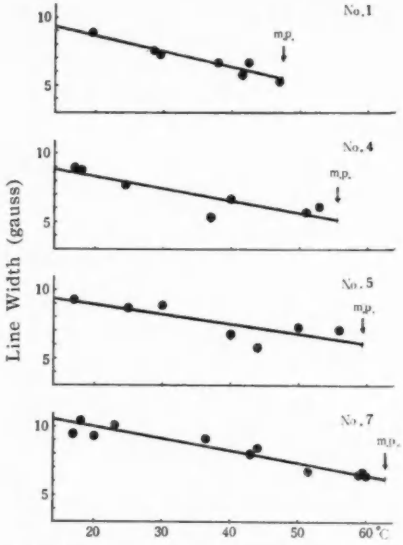

Fig. 3. Temperature dependence of the line widths. $(\Delta H)_{II}$

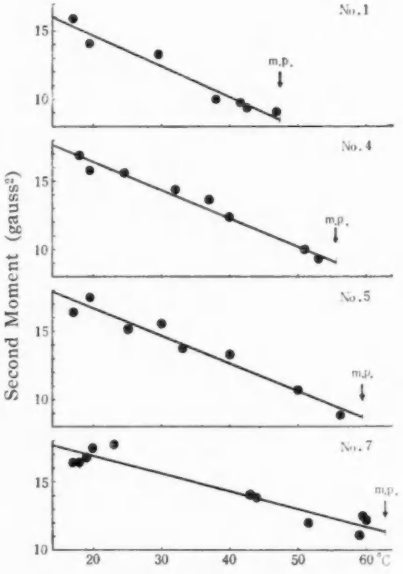

Fig. 4. Temperature dependence of the second moments. $\langle \Delta H^2 \rangle_{II}$

The experimental results of $(\Delta H)_{II}$, X_{II} and $\langle \Delta H^2 \rangle_{II}$ at 19°C and V_{II} are listed in Table I, and the data of temperature dependence depicted in Figs. 3, 4, 5 and 6, where the straight lines are obtained from about ten adequate points for each sample

TABLE I

Samples	m. p. °C	$(\Delta H)_{II}$* gauss	$\langle \Delta H^2 \rangle_{II}$* gauss^2	X_{II}* %	V_{II} kcal./mol.
No. 1	47.7	8.8	14.1	92.9	3.6
2	50.4	9.7	15.7	96.7	—
3	52.9	9.6	16.0	96.7	—
4	55.6	9.8	15.8	96.3	2.8
5	59.4	10.2	17.1	98.0	2.4
6	60.8	10.2	17.0	97.6	—
7	62.8	10.5	16.6	97.0	2.5

* at 19°C

by the method of least squares, and the suffix II refers to the data for the component II.

Findings in Table I and Figs. 3, 4, 5 and 6 may be summarized as follows: first, there seems to be intimate correlation between the nuclear magnetic resonance data, such as $(\Delta H)_{II}$ and $\langle \Delta H^2 \rangle_{II}$, and the composition of the samples; second, $(\Delta H)_{II}$ values are reduced gradually with temperature and become about 6 gauss just below the melting point; third, X_{II} values are not much reduced with temperature; lastly, the magnitudes of the activation energies V_{II} are almost equal to one another among the samples with the value of 2—3 kcal./mol.

According to the first finding, $(\Delta H)_{II}$ and $\langle \Delta H^2 \rangle_{II}$ values for the samples with higher melting point are larger than those with lower melting point. This may suggest that the component II in the samples with higher melting point is less motional than that in those with lower melting point. In another words, line-width data of the component II may be useful for the qualitative analysis of the melting point.

The second finding on the temperature dependece of the line-width of component II seems valuable to answer the question, "What kind of molecular motion would occur prior to melting of linear polymers?" Information we have had so far for this problem by any method such as dielectric measurements is rather insufficient.[7] According to the present results, as seen in Fig. 3, the molecular motion in component II increases with temperature, but the magnitude of this motion, of vibrational or diffusional nature, appears to be small* and the molecular state to be relatively close to the rigid lattice state up to melting.

7) R. F. Boyer, *J. Appl. Phys.*, **25**, 825, (1954).

* Magnetic dipolar broadening of the line-width value is easily reduced to one half or one third of the rigid lattice value by any molecular rotation[8]. In the present results, the $(\Delta H)_{II}$ values are ranged around 6 to 10 gauss, which are compared with the rigid value of 15 gauss.

As shown by one of the present authors,[9] the difference in the line-width, and consequently in the correlation time, among the samples with the same magnitude of V is attributed to the difference in the degree of polymerization among the samples. Thus the difference in line-width or correlation time among the samples No. 1, 4, 5 and 7 seems to be attributed to the difference in their molecular dimensions.

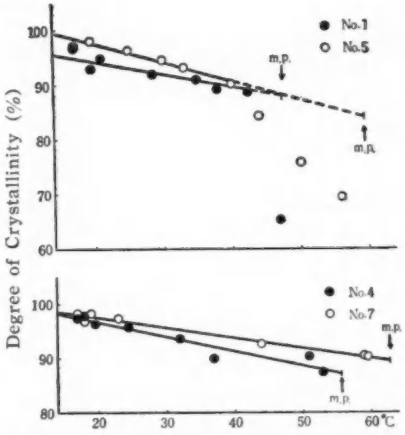

Fig. 5. Temperature dependence of the degree of crystallinity. X_{II}%.

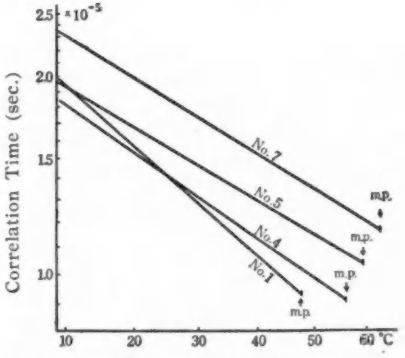

Fig. 6. Temperature dependence of the correlation time. $1/\nu_c$.

It is noted, especially, that the degree of crystallinity, X_{II}, is not much reduced prior to melting. This will suggest that the molecules maintain relatively rigid configurations between the nuclear nearest neighbors up to melting.

8) E. R. Andrew, *J. Chem. Phys.*, **18**, 607 (1950).

9) S. Fujiwara, to be published in this Bulletin.

Throughout the cases of the experiments, it was also found that both line-width and second moment values kept sizable magnitudes up to just below the melting point. These findings are very prominent and in contrast to the result on cyclohexane, where rapid decrease of the second moment occurs 130°C prior to melting.[10]

Namely, in the latter case, the molecular reorientation with fixed center of mass occurs 130°C prior to melting, whereas in the former, the molecules are relatively static up to just below the melting point.

The degree of crystallinity of microcrystalline paraffin waxes is much smaller than that of oil paraffins, and is about 90% at room temperature. This result perhaps shows the segments in microcrystalline waxes are much smaller and easier to diffuse in the sample than that in oil paraffins.

Many thanks are due to Mr. Kenzo Fusegawa of Dai Nippon Seiro Co., who gave us the samples used in the present experiments.

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10) E. R. Andrew and R. G. Eades, *Proc. Roy, Soc.*, **A 216**, 398 (1953).

Stoichiometric Investigations of the Liquid State IV[*1]. Viscosity of Associated Liquid*

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(Received May 9, 1957)

It is well known that viscosity of pure liquid is generally expressed by eq. (1), according to Eyring's absolute reaction rate theory.

$$\eta = \frac{hN}{V} e^{\Delta F^*/RT} \tag{1}$$

where h is Planck's constant, N, Avogadro's number, ΔF^*, standard free energy of activation per mole, and

$$\Delta F^* = \Delta H^* - T\Delta S^* \tag{2}$$

Ep. (1) can be rewritten as eq. (3)

$$\eta = \left[\left(\frac{hN}{V}\right)e^{-\Delta S^*/R}\right]e^{\Delta H^*/RT} \tag{3}$$

where ΔH^*, standard energy of activation per mole and ΔS^* standard entropy of activation per mole.

In general, the temperature variance of the frequency factor of eq. (3) is very little compared with that of the exponential energy factor. Therefore, eq. (1) can be rewritten as eq. (4).

$$\eta = Ae^{E_{vis}/RT} \tag{4}$$

where E_{vis} is activation energy per mole for viscous flow.

In the case of associated liquid, Eyring and others[1] thought that E_{vis} depends upon temperature because of the destruction of hydrogen bond with increase in temperature. They showed that E_{vis} decreases with the increase of temperature in the case of water, ethylene glycole and glycerin, but said nothing about typical associated liquid such as saturated alcohol and fatty acid. Thereafter, investigations concerning the viscosity of associated liquid[2-4] are unexpectedly few. The present author discussed the reasonableness of classifying associated liquid into two kinds from analysis of sound velocity in liquid in the previous paper[5]. In the other paper[6], an empirical method for calculating the degree of association of associated liquid of the first kind was proposed. In this paper, detailed analysis of viscosity of associated liquid is presented.

Method and Result

E_{vis} can be calculated by eq. (5) from eq. (4).

*1 The third paper of this series, *Bull. Chem. Soc. Japan*, **27**, 306 (1954).

*2 Present address: Research Laboratory, Dai-Nippon Celluloid Co., Sakai, Osaka.

1) S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes" p. 477 (1941).
2) O. Kimura, *J. Chem. Soc. Japan*, **63**, 1068 (1942).
3) Y. Wada, *J. Phys. Soc. Japan*, **4**, 136 (1949).
4) L. Grunberg, and A. H. Nissan, *Trans. Faraday Soc.*, **45**, 125 (1949).
5) K. Tyuzyo, This Bulletin **27**, 306 (1954).
6) K. Tyuzyo, *Kolloid-Z*, **131**, 40 (1953).

TABLE I

E_{vis}, A AND DIELECTRIC CONSTANT OF ASSOCIATED LIQUID

Substance	E_{vis} (Kcal/mole)	A	Range (°C)	Dielectric constant	temp. / freq.
	saturated fatty acid				
formic acid	3.35	5.63×10^{-5}	8～100[a]	58.5	16 / 0
acetic acid	2.66	1.29×10^{-4}	31～119	6.17	20 / 0
propionic acid	2.47	1.63 〃	17～137	3.1	room. temp. / λ=83 cm
n-butylic acid	2.81	1.25 〃	18～156	3.0	room. temp. / 0
n-valeric acid	3.27	8.24×10^{-5}	16～90[a]		
n-caproic acid	3.57	7.06 〃	〃		
n-heptylic acid	3.84	6.05 〃	20～90		
n-caprylic acid	4.18	4.07 〃	50～90		
n-pelargonic acid	4.58	3.11 〃	20～90		
n-capric acid	4.76	2.71 〃	50～70		
n-lauric acid	5.10	2.69 〃	50～160		
n-myristic acid	5.34	2.46 〃	60～90		
n-palmitic acid	5.54	2.40 〃	70～95		
n-stearic acid	5.74	2.26 〃	70～98		
	saturated aliphatic alcohol				
methyl alcohol	2.61	6.92×10^{-5}	0～60	31.8	19 / 0
ethyl alcohol	3.57	2.74 〃	10～90	25.8	20 / 0
propyl alcohol	4.39	1.24 〃	10～96	21.3	19 / 0
n-butyl alcohol	4.64	1.07 〃	10～114	19.1	19 / 0
n-amyl alcohol	5.20	7.30×10^{-6}	12～80	16.0	room temp. / 0
n-hexyl alcohol	5.40	4.97 〃	25～50		
n-heptyl alcohol	5.76	3.63 〃	25～90		
n-octyl alcohol	5.99	3.13 〃	15～90		
n-cetyl alcohol	6.86	2.74 〃			
	others				
hydrogen cyanide	1.64	1.15×10^{-4}	0～20	ca. 95	21 / λ=84cm.
hydrogen fluoride	1.69	1.19 〃	−90～20	83.6	0 / 0
formamide	4.10	3.07×10^{-5}	0～120	111.5	20 / 0
water				81	19 / 0
ethylene glycole				38.7	
glycerine	varies with temperature	varies with temp.		63	−50 / 0
aniline				7.21	20 / $0\sim4\times10^9$
ammonia				25	−77.7 / 0

a) $\log\eta$ vs. $1/T$ somewhat curves.

$$E_{vis}=\frac{\mathrm{d}\ln\eta}{\mathrm{d}(1/T)}\times R \qquad (5)$$

When $\ln\eta$ vs. $1/T$ is linear, E_{vis} it constant, independent of the temperature. In Table I, E_{vis} and A of several kinds of associated liquid are shown. In the table, dielectric constant is also shown, to be used aftewards*.

* Viscosity and dielectric constant are adopted from the following literatures.
International Critical Tables; *Landolt-Börnstein, Phys.-Chem Tabellen*; D'Ans-Lax, *Taschenbuch für Chemiker und Physiker.*

Discussion

(1) Classification of associated liquid.—It is clear from Tab. I, that associated liquid can be distinguished clearly into two classes. E_{vis} of one class is constant, independent of the temperature, that of the other class decreases with increase in temperature. Several examples are shown in Fig. 1. In the figure, formic acid and methyl alcohol belong to the former, whereas water and aniline to the latter. Therefore, the author proposes to classify associated liquid into two groups, as shown below. This method of classification is almost consistent with that based on sound velocity discussed in the previous paper[5]. This method is also almost completely consistent with that of Ewell and others[7].

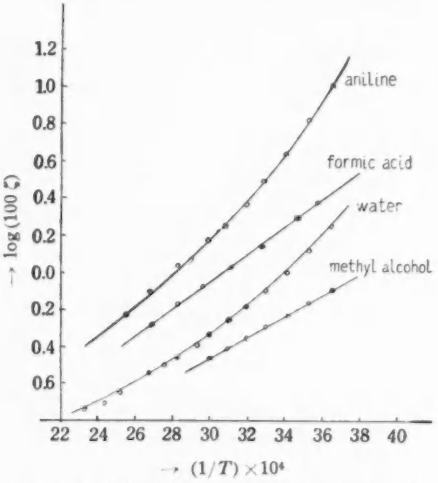


Fig. 1. Viscosity vs. temperature of several associated liquids.

a) *Associated liquid of the first kind*

E_{vis} is constant, independent of the temperature as high as the boiling point. In this class of associated liquid, associated bodies can be distinguished clearly from one another, owing to the strong hydrogen bond. Each associated body flows as a unit without destroying hydrogen bond on viscous flow.

Example: saturated fatty acid, saturated alcohol*, hydrogen cyanide, hydrogen flouoride, formamide.

b) *Associated liquid of the second kind*

E_{vis} decreases with increase in temperature. In this class of associated liquid, there are no distinguishable associated bodies owing to the weak hydrogen bond. The whole liquid makes a three-dimensional network structure and hydrogen bond is destroyed on viscous flow. This flow mechanism is consistent with that of Eyring and others[1].

Examples: water, ethylene glycole, glycerin, ammonia, aniline.

In Table II, values of hydrogen bond energy are shown[10]. In general, hydrogen bond energy of the associated liquid of the first kind is somewhat larger than that of the associated liquid of the second kind except hydrogen cyanide. However, the idea that this small difference in hydrogen bond energy causes the above-mentioned fundamental difference of the behavior of viscous flow is quite questionable. Details of hydrogen bond energy may be discussed in the next paper of this series.

TABLE II
ENERGY OF HYDROGEN BOND

Substance	State	Method	Energy (Kcal./mole)
HF	gas	vapor density	6∼10
Fatty acid	gas	vapor density	7∼9
Alcohol	liquid	heat of vapor	6∼7
	gas	heat conduct.	7∼8
HCN	gas	vapor density	3∼4
Water	s, l, g	heat of vapor	4∼5
		second virial	
	gas	second virial	3∼4
Ammonia	liquid	dielectric const.	6
	solid	heat of sublim.	1.3
Acetamide	solid	heat of sublim.	3.5
Aniline		partition coeff. (CCl_4—H_2O)	1.93

(2) E_{vis} of unassociated liquid.—In this paragraph, E_{vis} of unassociated liquid is treated in outline, in order to clarify the structure and the degree of association of associated liquid of the first kind.

a) *E_{vis} of n-paraffin*

Relation between E_{vis} and molar volume (V)* at room temperature of n-paraffin is shown in Fig. 2. In this figure, E_{vis} is

7) R. H. Ewell, J. M. Harrison and L. Berg, *Ind. Eng. Chem.*, **36**, 871 (1944).

* In some papers[8,9], it is said that E_{vis} of fatty acid and alcohol varies with temperature but this variance is little. Moreover, E_{vis} is dependent upon temperature with some degree even in n-paraffin. Therefore, it is reasonable to regard E_{vis} of fatty acid and alcohol as being independent of temperature at this time.

8) A. Bondi, *J. Chem. Phys.*, **14**, 591 (1946).

9) T. A. Litovitz, *J. Chem. Phys.*, **20**, 1088 (1952).

10) L. N. Fergusson, "Electron Structures of Organic Molecules", p. 57 (1952); S. Seki, *Chem. and Chem. Ind., Japan*, **6**, 217 (1953).

* Molar volume at 50°C is adopted in the case of higher members.

calculated from recent data[11]. From this figure, eq. (6) is obtained.

$$E_{vis}(\text{Kcal/mole}) = 5.18 \log V(\text{cc/mole}) - 9.07 \quad (6)$$

Eq. (6) is applicable at least to the range of V from 80 to 570. It is natural that eq. (6) is not applicable when viscous flow becomes segmental because of independence of E_{vis} upon V in such a region.

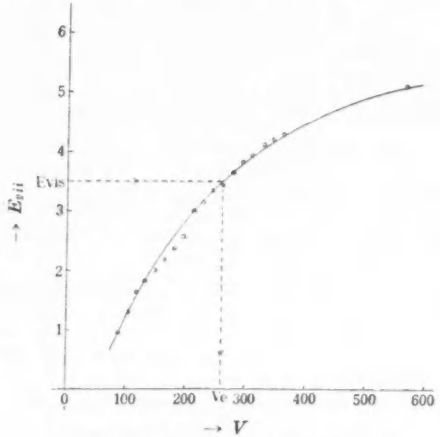


Fig. 2. E_{vis} vs. V of n-paraffins.

b) *E_{vis} of unassociated liquid except n-paraffin*

E_{vis} of unassociated liquid is compared with that of n-paraffin. Results are summarized as follows.

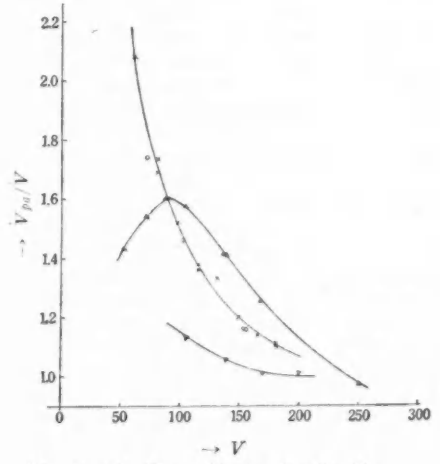


Fig. 3. V_{pa}/V vs. V of polar liquids.
×: ester ○: ketone
□: alkyl-chloride ▽: ether

11) "Selected Values of Properties of Hydrocarbons", (National Bureau of Standards), 1947.

i) Compared with the same value of V, E_{vis} of branched aliphatic compounds is almost the same as that of linear aliphatic compound within ±10%. This means that the influence of branching upon E_{vis} is very small.

ii) Compared with the same value of V, E_{vis} of polar compounds is greater than that of n-paraffin, but this difference decreases rapidly with higher homologous members and is almost zero in the region of V greater than 200. Fig. 3 shows this relation. In the figure, abscissa is V and ordinate is the ratio of V of n-paraffin having the same value as E_{vis} of the polar compound (hereafter, V_{pa}) to V of the polar compound.

iii) Compared with the same value of V, E_{vis} of cyclic compounds having no substituted group is much greater than that of n-paraffin. Namely, $V_{pa}/V \fallingdotseq 2.0$, independent of polarity. This is shown in Table III. It is clear from this fact that rigidity of structure has great influence upon viscous flow.

TABLE III
V_{pa}/V OF CYCLIC COMPOUND WITH NO SUBSTITUTED GROUP

Substance	V	E_{vis}	V_{pa}/V
Benzene	89	2.52	2.0
Cyclohexane	108	2.90	1.9
Dioxane	85.4	2.88	2.4
Thiophene	78.7	2.17	1.9
Pyridine	80.8	2.48	2.2
Cycopentane	94	1.88	1.4
Biphenyl	155	3.61	1.8

iv) V_{pa}/V decreases rapidly with increase of the number of substituted group of cyclic compound. Some examples are shown in Table 4.

TABLE IV
INFULENCE OF SUBSTITUTED GROUP OF CYCLIC COMPOUND UPON V_{pa}/V

Substance	V	E_{vis}	V_{pa}/V
Benzene	89	2.52	2.0
Toluene	106	2.90	1.9
Ethyl benzene	122	2.17	1.3
n-Butyl benzene	156	2.03	0.9
Cyclohexane	108	2.90	1.9
Methyl cyclohexane	128	2.50	1.4
1,2-Dimethyl cyclohexane	144	2.35	1.2

(3) Entropy of activation, ΔS^* of viscous flow of unassociated liquid—ΔS^* is calculated as follows.

In eq. (4),

TABLE V
ENTROPY OF ACTIVATION OF VISCOUS FLOW OF UNASSOCIATED LIQUID

Substance	A	ΔS^* (E.U./mole)
Methane	1.09×10^{-4}	−0.93
Carbon tetrachloride	1.23 〃	−2,21
Benzene	8.71×10^{-5}	−1.32
Cyclohexane	6.76 〃	−1.21
Dioxane	9.16 〃	−1.40
Carbon disulfide	4.25×10^{-4}	−3.71
Toluene	1.55 〃	−2.83
Acetone	1.95 〃	−2.56
Methyl ethyl ketone	1.55 〃	−2.76
Ether	1.55 〃	−2.71
n-Ethyl valeriate	1.32 〃	−3.20
n-Butyl valeriate	1.12 〃	−3.23
n-Butyl butylate	1.32 〃	−3.37
n-Amyl n-butylate	1.20 〃	−3.38
Ethyl chloride	2.29 〃	−2.80
Ethylene chloride	2.95 〃	−3.10
Chloroform	2.82 〃	−3.46
n-Propyl chloride	1.32 〃	−2.14
Ethyl bromide	1.55 〃	−2.13
Thiophene	1.62 〃	−2.40
Pyridine	1.41 〃	−2.20
Ethane	2.49 〃	−3.06
Propane	2.69 〃	−3.57
n-Butane	1.94 〃	−3.19
n-Pentane	1.36 〃	−2.76
n-Hexane	1.34 〃	−2.99
n-Heptane	1.33 〃	−3.20
n-Octane	1.29 〃	−3.34
n-Nonane	1.23 〃	−3.44
n-Decane	1.15 〃	−3.45
n-Undecane	7.13×10^{-5}	−2.68
n-Dodecane	6.70 〃	−2.69
n-Tridecane	6.11 〃	−2.64
n-Tetradecane	6.40 〃	−2.87
n-Pentadecane	5.53 〃	−2.69
n-Hexadecane	4.92 〃	−2.57
n-Heptadecane	4.72 〃	−2.59
n-Octadecane	4.13 〃	−2.44
n-Nonadecane	4.34 〃	−2.65
n-Eicosane	3.65 〃	−2.40
	Average	−2.9

$$A=\frac{hN}{V}e^{-\Delta S^*/R} \tag{7}$$

Inserting numerical values of R, h, N into eq. (7),

$$\Delta S^*=-11.1-4.6\log(VA) \tag{8}$$

ΔS^* is easily calculated from eq. (8). Results are shown in Table V. It is clear from the table that

a) ΔS^* is almost constant and approximately equal to −2.9 E.U./mole, irrespective of polar and non-polar liquid except spherical molecules.

b) ΔS^* of spherical molecules is abnormal and approximately equal to −1.0∼−2.0 E.U./mole. Abnormality of E_{vis} of spherical molecules is already shown by Eyring and others[12]. It is very interesting that ΔS^* of spherical molecules is also abnormal as is shown above.

(4) **Method for calculating degree of association of associated liquid of the first kind.**—As is shown in (1), hydrogen bond is not destroyed on viscous flow of associated liquid of the first kind. Then, it is probable that viscous flow of this kind of associated liquid is quite analogous to that of unassociated liquid. Therefore, degree of association is calculated by the following method, on trial[6].

a) *Method based on* E_{vis}

It is already shown that relation between E_{vis} and V at room temperature for *n*-paraffin is as Fig. 2 and expressed by eq. (6). V of *n*-paraffin, corresponding to E_{vis} of associated liquid of the first kind, can be obtained from Fig. 2 or eq. (6). This is termed V_e. Then, degree of association is calculated by eq. (9). Degree of association calculated by eq. (9) is termed as n_e.

$$n_e=V_e/V \tag{9}$$

where V is normal molar volume.

n_e is shown in Table VI. n_s in the table is referred to in the next paragraph. Degree of association from literature is also shown in the table. The following points are clear from the table.

i) n_e is surprisingly consistent with value from literature.

ii) It is possible to calculate easily the degree of association which has not been known until now.

iii) The n_e values for formic and acetic acid are much greater than 2 on the contrary to propionic acid and higher members of saturated fatty acid. This means that the state of association is different from each other. Investigation of the state of association in the pure liquid state is very rare. But according to the investigation of infra-red spectra by Batuev[14], formic and acetic acid make linear chain association and propionic acid and higher members make ring-like dimer association,

12) H. Eyring, *J. Chem. Phys.*, **4**, 283 (1936); R. H. Ewell and H. Eyring, *J. Chem. Phys.*, **5**, 726 (1937).

13) W. Kauzmann and H. Eyring, *J. Am. Chem. Soc.*, **62**, 3113 (1640).

14) M. I. Batuev, *Chem. Abst.*, 1936, 2994, 2995, 6154(1947).

TABLE VI

DEGREE OF ASSOCIATION OF THE ASSOCIATED LIQUID OF THE FIRST KIND

Substance	V	V_e	n_e	V_s	n_s	n	Lit. of n
	saturated fatty acid						
formic acid	37.7	251	6.7	303	8.0	Polymer	Batuev[14]
acetic acid	57.2	194	3.4	132	2.3	〃	〃
propionic acid	74.6	179	2.4	105	1.4	2	Batuev[14] &
n-butylic acid	91.9	206	2.2	136	1.5	〃	Gibling[15]
n-valeric acid	108.9	245	2.2	201	1.9	〃	〃
n-caproic acid	125.0	268	2.1	249	2.0	〃	〃
n-heptylic acid	141.9	298	2.1	315	2.2	〃	〃
n-caprylic acid	158.6	337	2.1	426	2.7	〃	〃
n-pelargonic acid	174.5	400	2.3	541	3.1	〃	〃
n-capric acid	196.2 (50°C)	433	2.2	608	3.1	〃	〃
n-lauric acid	229.8 (50°C)	516	2.2	655	2.8	〃	〃
n-myristic acid	270.4 (50°C)	603	2.2	695	2.6	〃	〃
n-palmitic acid	304.6 (50°C)	668	2.2	725	2.4	〃	〃
n-stearic acid	338.9 (50°C)	724	2.1	758	2.2	〃	〃
	saturated aliphatic alcohol						
methyl alcohol	40.5	191	4.7	245	6.0	5.0	Eucken[16]
ethyl alcohol	58.3	268	4.6	605	10.0	4.7	〃
propyl alcohol	75.0	367	4.9	1170	15.6	4.7	〃
n-butyl alcohol	92.0	411	4.5	1690	18.4	—	—
n-amyl alcohol	107.7	575	5.3	2610	24.3	—	—
n-hexyl alcohol	125.2	617	4.9	3390	27.0	—	—
n-heptyl alcohol	140.7	741	5.3	4240	29.8	—	—
n-octyl alcohol	156.7	813	5.2	5660	35.6	—	—
n-cetyl alcohol	(285)	1202	4.3	6780	23.0	—	—
	others						
hydrogen cyanide	38.5	119	3.1	148	3.8	3	Pauling[17]
hydrogen fluoride	20.4	112	6.0	143	7.1	6∼10	Bauer et al[18]
formamide	39.9	331	8.3	553	13.8	—	—

as is generally recognised. This is quite consistent with the above result. The following paragraph (5) may be refered to about this fact.

iv) n_e of saturated aliphatic alcohol is 5, independent of the carbon number. This result is consistent with Eucken's[16].

v) V_e and n_e of stearic acid are 724 and 2 respectively. V_e and n_e of octyl alcohol are 813 and 5.2 respectively. These are quite reasonable values. But n_e of cetyl alcohol is 4.3 and is slightly smaller than 5. From these facts, it can be supposed that the limit of eq. (6) exists between 810 and 1200 of V. Namely, it can be supposed that segmental flow occurs at least in the range of carbon numbers of greater than 50 for *n*-paraffin. This result is different from Kauzmann and Eyring's[13].

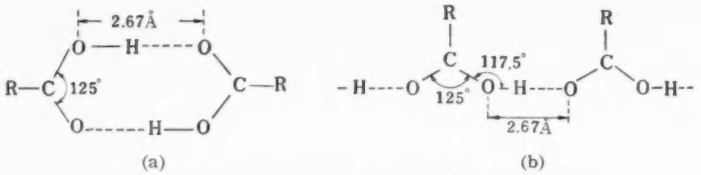
vi) No conclusive method has been so far found for determining degree of association in the pure liquid state. Moreover, accessible methods are all roundabout and complex. Although the above method is quite empirical, it is very effective under such a circum-stance.

b) *Method based on* ΔS^*

It is shown in Paragraph (3) that ΔS^* of unassociated liquid is about -2.9 EU./mole except in the case of spherical molecules. Inserting $\Delta S^* = -2.9$ in eq. (8), eq. (10) is obtained. V from this is expressed as V_s.

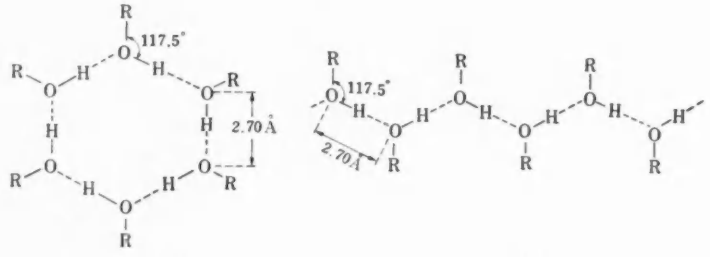
$$V_s = 1.70 \times 10^{-2}/A \qquad (10)$$

15) T. W. Gibling *J. Chem. Soc.*, 665 (1942).
16) A. Fucken, *Z. Elektrochem.*, **52**, 255 (1948).
17) L. Pauling, *Nature of Chemical Bond*, p. 275 (1939).
18) S. H. Bauer, J. Y. Beach and J. H. Simons, *J. Am. Chem. Soc.*, **67**, 757 (1945).



(a) (b)

Fig. 4. Associated state of saturated fatty acids.



(a) (c)

Fig. 5. Associated state of saturated aliphatic alcohols[20].

In eq. (10), A is obtained from temperature variance of viscosity by eq. (4). Degree of association, n_s may be calculated by eq. (11).

$$n_s = V_s/V \tag{11}$$

where V is normal molar volume. The n_s values are shown in Table VI.

It is clear from the table that n_s is fairly consistent with values from literature, although the agreement is not so good as for n_e. However, n_s of saturated alcohol is quite different from the observed value. The reason for this is not clear, but it may be supposed that polydispersity of association of saturated alcohol[19] in the liquid state may have some influence upon this point.

(5) **Associated state of associated liquid of the first kind.**—In this paragraph, association state of associated liquid of the first kind is discussed in detail.

(a) *formic acid*

According to (4), degree of association of formic acid is 7 to 8. If association state is a ring-like dimer as is shown in Fig. 4-(a), $V_{pa}/V \fallingdotseq 2.0$ from (2)-(b), because this state is non-branched ring. But as

$$E_{vis}=3.35,\ V_{pa}/V=251/2\times 37.7=3.3\neq 2.0$$

This is unreasonable. Moreover, the dielectric constant is very great (58.5 as is shown Table I), and liquid viscosity is almost the same as that of valeric acid, which is higher than acetic and propionic acid. Considering these facts, it is reasonable that association is chain association as is shown in Fig. 4-(b).

(b) *acetic acid*

According to (4), n is about 3. If association state is ring-like dimer as is shown in Fig. 4-(a), $V_{pa}/V=194/2\times 57.2=1.7$. But V_{pa}/V of such a slightly branched ring compound is 1.2 to 1.5 from (2)-(b). Moreover, dielectric constant is comparatively small (6.17 as is shown in Table I) and viscosity is higher than that of propionic acid. Considering these facts, it is reasonable that association state is the mixture of dimer association and chain association as is shown in Fig. 4-(a), (b).

(c) *saturated fatty acid higher than propionic acid.*

According to (4), $n=2$. If association state is a perfect ring-like dimer as is shown in Fig. 4-(a), $V_{pa}/V=1.0$. V_{pa}/V of such a branched ring compound is 1 from (2)-(b). Both these are quite consistent. Moreover, dielectric constant is small (2 to 3 as is shown in Table I). Considering these facts, it is reasonable to assume that association state is dimer association as is shown in Fig. 4-(a).

(d) *saturated alcohol*

According to (4), $n=5$. If association state is ring-like association of $n=6$, $V_{pa}/V=1.0$ from (2)-(b). However, $V_{pa}/V = \frac{5}{6} \neq 1$. Moreover, the dielectric constant

19) K. L. Wolf and R. Wolff, *Angew. Chem.*, **61**, 191 (1949).

20) L. Pauling, "Nature of Chemical Bond", p. 285 (1939).

is the greatest for methyl alcohol (31.8) and becomes smaller with increasing number of carbon atom. However, dielectric constants of all these compounds are much greater than those of fatty acid higher than propionic acid. Therefore, it is reasonable that association state is chain association as is shown in Fig. 5-(b). Eucken concluded chain association of $n=5$ for lower alcohols[16]. This conclusion is quite consistent with the above.

(e) *hydrogen fluoride*

According to (4), $n=6\sim7$ and is consistent with the value from literature[18]. If association state is unbranched ring-like association, $V_{pa}/V=2.0$ from (2)-(b). Then from observed E_{vis}, $V_{ag}=V_{pa}/2.0=122/2.0=61$. Therefore. $n=61/20.4\fallingdotseq3$. This value is unreasonable, on the basis of the ring-like structure and is not consistent with the observed value. Moreover, dielectric constant is very great (83.6 as is shown in Table I). When these facts are considered, it is reasonable that association state is chain association of $n=6\sim7$ as is shown in Fig. 6.

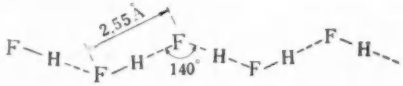


Fig. 6. Association state of hydrogen fluoride[21].

(f) *Hydrogen cyanide*

According to (4), $n=3$ and is consistent with the value from literature[17]. If association state is unbranched ring-like association, $V_{pa}/V=2.0$ from (2)-(b). Then from observed E_{vis}, $V_{ag}=V_{pa}/2.0=59.5$. Therefore, $n=59.5/38.5=1.6$. This value is unreasonable, on the basis of the ring-like structure and is not consistent with the observed value. Moreover, dielectric constant is very great (ca. 95 as is shown in Table I). When these facts are considered, it is reasonable that association state is chain association of $n=3$.

(g) *Formamide*

According to (4), $n=8\sim14$. If association state is unbranched ring-like association, $V_{pa}/V=2.0$ from (2)-(b). Then from observed E_{vis}, $V_{pa}/V=V_{pa}/2=165.5$. Therefore, $n=165.5/39.9\fallingdotseq4$. A decisive conclusion cannot be made because of the lack of the observed value. The dielectric constant is very great (111.5 as is shown in Table I). Considering these, it is probable that association state is chain association of $n=8\sim14$.

(6) Association degree of alcohols, hydrogen fluoride and hydrogen cyanide from (4) is quite consistent with the observed degree. On the other hand, these liquids are chain association and their dielectric constants are great as is discussed in (5). If the non-polar part is extremely great compared with the polar part of polar liquid, $V_{pa}/V=1.0$ according to (2). But, in the case of the above compounds, polar part is not always extremely small compared with non-polar part. In spite of this, $V_{pa}/V_{ag}=1$ as is shown in (5). A complete explanation of this fact can not be given at present.

Summary

(1) It is concluded from analysis of viscous flow that associated liquid can be classified as follows.

The first kind: Associated bodies can be distinguished clearly from one another, owing to the strong hydrogen bond. Each associated body flows as a unit in the case of viscous flow.

Examples: saturated fatty acid, saturated aliphatic alcohol, hydrogen fluoride, hydrogen cyanide.

The second kind: There are no associated bodies which are distinguished clearly from one another, owing to the weak hydrogen bond. The liquid makes overall three-dimensional network structure. Hydrogen bond is destroyed in the case of viscous flow.

Examples: water, ethylene glycole, glycerin, ammonia.

(2) Influence of branching, polar group and ring upon activation energy of viscous flow of unassociated liquid is discussed.

(3) It is found that entropy of activation of viscous flow of unassociated liquid is constant and equal to ca. −2.9 E.U./mole with some exceptions.

(4) The method for calculating degree of association of associated liquid of the first kind from energy and entropy of activation of viscous flow is presented.

(5) Association state of the first kind liquid is discussed in detail.

The author expresses his sincere thanks to Professor Dr. N. Sata and Mr. M. Okuyama of Osaka University for their kind instruction.

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21) L. Pauling, "Nature of Chemical Bond", p. 276 (1939),

Studies on Organic Reagents for Inorganic Analysis. II[1]. *Spectrophotometric Study on Phenylfluorone Chelates of the Fourth Group Metal Elements**

By Hirotoshi SANO

(Received March 26, 1957)

Introduction

The study on the nature of the reaction of metals with organic reagents is very important for the purpose of selecting or sythesizing an organic reagent useful for the colorimetric determination of a metal. There are two groups of organic reagents used for this purpose; (1) For the first group, e. g. ethylene diamine or EDTA, the light absorption is attributed to the electronic transition from nonbonding orbital to antibonding orbital in a central metal atom as is usual with simple inorganic complexes[2]. (2) Most organic dyes used as the reagents for the colorimetric determination of metals belong to the second group, and their color changes are, as is stated in the following, ascribable to the shift of the $\pi \rightarrow \pi^*$ absorption spectra of the organic system caused by the bond formation between the metal and the donor atom of the reagent. For the investigation of such a color change, it is necessary to understand the general theory of light absorption of organic dyes. As is well known, there are essentially three methods, valency bond method, molecular orbital method, and free electron gas model method. Among them, the last method is much simpler than the others, and moreover its utility is almost comparable to that of the molecular orbital (L. C. A. O.) method which needs the cumbersome procedure of solving secular equations[3].

Recently, the free electron gas model method has been used extensively for calculating the wavelength of the absorption maximum in the spectrum of conjugated organic compound[4-11]. In particular, H. Kuhn has applied this method successfully to various organic dyes[7-11]. According to him, the electron gas in the cases of polyenes, unsymmetrical cyanines, oxanoles etc. suffers a disturbance, and the π-electrons are considered to be placed in a one-dimensional potential having a sine curve periodicity[8]. The wavelength of absorption maximum is expressed by the following equation.

$$\frac{1}{\lambda_{max}} = \frac{V_0}{h \cdot c}\left(1 - \frac{1}{N}\right) + \frac{h}{8mc} \cdot \frac{N+1}{L^2} \qquad (1)$$

where, L is the length of the path of the free π-electron measured along the conjugated chain, N, the number of π-electrons, m, the mass of the electron, c, the velocity of light, h, Planck's universal constant, and V_0 the amplitude of the sine-shaped potential along the chain. In the present study, this model was adopted to examine the relation between the nature of the chelating bond and the color change of reagent. 2,3,7-Trihydroxy-9-phenyl-fluorone was chosen as a suitable organic reagent, and is an excellent compound for such a purpose because of its symmetric structure and of the high stability of its chelates with fourth group metals.

Experimental and Results

Reagents and Apparatus. — The ethanolic solution of phenylfluorone (1 ml. contains 0.6 mg. of phenylfluorone*) was prepared by dissolving 0.120 g. of phenylfluorone in 150 ml. of ethyl alcohol containing 1 m. of 5.0 N hydrochloric acid, and the resulting solution was diluted to 200 ml. with ethyl alcohol.

* This study was presented at the Meeting of the Chemical Society of Japan on Oct. 28, 1956 (Symposium on Chemistry of Complex Compounds).

1) K. Kimura and H. Sano, This Bulletin, **30**, 80 (1957).
2) H. Yamatera, *J. Inst, Polytechnics Osaka City Univ.*, **5**, 163 (1956).
3) H. H. Jaffé, *J. Chem. Phys.*, **20**, 1646 (1952); ibid, **21**, 1287 (1953).
4) N. S. Bayliss, *J. Chem. Phys.*, **16**, 287 (1948).
5) J. R. Platt, ibid., **17**, 484 (1949).
6) W. T. Simpson, ibid, **17**, 1218 (1949).
7) H. Kuhn, *Helv. Chim. Acta*, **31**, 1441 (1948); *J. Chem. Phys.*, **16**, 840 (1948).
8) H. Kuhn, *Z. Elektrochem.*, **53**, 165 (1949); *J. Chem. Phys.*, **17**, 1198 (1949).
9) H. Kuhn, *Helv. Chim. Acta*, **34**, 2371 (1951); *Z. Elecktrochem.*, **55**, 220 (1951).
10) W. Huber, H. Kuhn and W. Huber, *Helv. Chim. Acta*, **36**, 1957 (1953).
11) H. Kuhn, *Z. Naturforsch*, **9a**, 989 (1954); *Z. Elektrochem.*, **58**, 219 (1954).

* Abbreviation of 2, 3, 7-trihydroxy-9-phenylfluorone.

Metal solutions were prepared from their oxides (germanium, titanium), chloride (tin), or oxychloride (zirconium), and then standardized gravimetrically. These stock solutions contain enough hydrochloric acid to prevent the hydrolysis of metals, being free from foreign metal ions. The working solution was prepared by diluting an aliquot of each stock solution with water or diluted hydrochloric acid.

Gum arabic solution was prepared by dissolving 0.5 g. of gum arabic in 200 ml. of water by warming. The solution was filtered when necessary.

Cyclohexanol. — Analytical grade reagent was used without further purification.

Absorbance values of the sample and the blank solution were measured with a Beckman spectrophotometer Model DU, using 1-cm. glass cells and distilled water as a reference solution. From the difference the absorption spectrum of the dye or the chelate was obtained.

Absorption spectra of dyes.—The solution whose absorption spectrum is shown in Fig. 1, contains 20 ml. of ethanol, and 0.6 or 1.2 mg. of phenylfluorone. The desirable acidity or basicity in the final solution was maintained by adding the appropriate volume of hydrochloric acid or aqueous potassium hydroxide solution. The solution was diluted to 50 ml. with water.

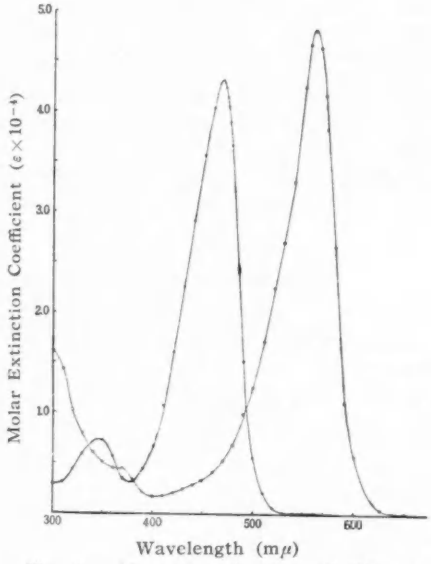


Fig. 1. Absorption Spectra of Phenylfluorone.
—●— in 0.5 N HCl solution
—○— in 1.0 N KOH solution

The absorption maximum of this dye is found at 468 mμ in an acidic solution, whereas it lies at 560 mμ in an alkaline solution. The value of molar extinction coefficient in alkaline solution might be slightly underestimated, because of the rapid fading of color due to the oxidation of the dye. With respect to the absorption maxima, the same results were obtained in the presence of either 5 ml. of cyclohexanol or gum arabic in the solution, although there was a slight difference in the detailed shape of their absorption curves. The solutions cause the precipitation of phenylfluorone in hydrochloric acic concentration below 0.04 N or in potassium hydroxide concentration below 0.01 N.

Reaction with Metals.—Phenylfluorone is one of the ortho-diphenol derivatives having the mesomeric group at its para position, which reacts remarkably with several metals, particularly titanium, germanium, zirconium, tin and thorium, giving striking color change[1,12].

Because the resulting complexes are insoluble in many solvents, the absorbance values were measured in the presence of a suitable stabilizer such as gum arabic or cyclohexanol*. The position and the intensity of absorption maximum is almost independent of the kind of the stabilizer used. On the other hand, the absorbance decreases with increasing acidity of solution. The effect of acidity is shown in Fig. 2. Beyond a certain value of acidity, the absorbance of chelate increases rapidly with the decrease in acidity. This specific value should be dependent on the stability of the chelate[13]. Then, the absorbance becomes nearly constant at lower acidity. In this range the molar extinction coefficients of the metal chelates are calculated, except for the case of titanium and tin chelate in which this range was not observed.

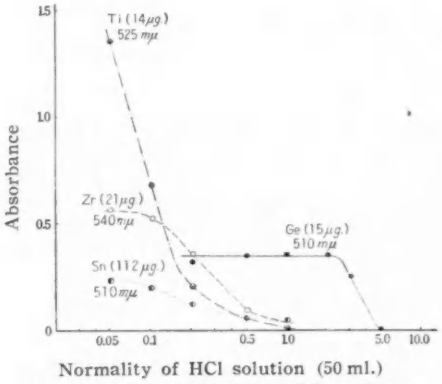


Fig. 2. Effect of Acidity. The solution contains 6 mg. of phenylfluorone, 20 ml. of ethanol, and 5 ml. of cyclohexanol or gum arabic solution in a final volume 50 ml.

* The light scattering effect of the colloidal particle on the absorption spectra is negligible for several hours after the preparation of the solution.

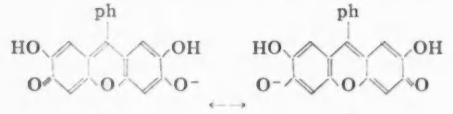
12) F. Ihmura, H. Sano and M. Asada; Unpublished report.

13) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds", New York Prentice-Hall, Inc. N. Y. (1952) p. 119.

The absorption sepectra of metal chelates with phenylfluorone were measured at various acidities, but the position of the absorption maximum was constant. The ratio of reagent to metal was obtained by the gravimetric analysis of the chelate precipitate. Their results are shown in Table I, together with the several properties of the metal. These results show that the value of ε_{max} is roughly proportional to the ratio of the reagent to the metal in each chelate complex. This fact suggests that the absorption spectra in this wavelength range depend on the $\pi \rightarrow \pi'$ transition of the conjugated π-electron in the ligand.

Discussion

The anionic phenylfluorone, which is responsible for the red-pink color observed in an alkaline solution, is considered to be resonating between two limiting structures, Ia and Ib, or IIa and IIb, each of which makes the same contribution to the normal state of the anion, because of its symmetrical structure. One form results from the other when the single and double



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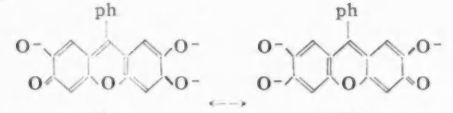
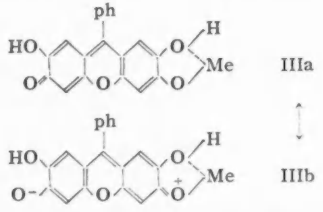


bonds of the conjugated chain change over. This shows that each conjugated C—C bond has an equal bond length and bond order as a rough approximation. The value of V_0 is, therefore, considered to be zero. Then, the equation (1) is expressed as follows.

$$\lambda_{max} = \frac{8mc}{h} \cdot \frac{L^2}{N+1} \qquad (2)$$

In the equation (2), N, m, c and h are constants. But L, the length of the conjugated chain, has a somewhat arbitrary character. In the present study, L was calculated from the observed wavelength of absorption maximum of the phenylfluorone anion, and this value was used in the following calculation and discussion*. For the phenylfluorone metal chelates the two limiting structures, IIIa and IIIb, are not equal in energy and they will not have equal contributions to the normal state, unlike the case of the symmetrical phenylfluorone anion. V_0 has, therefore, a difinite value.



On the other hand, if we conventionally apply the ionic-covalent resonance to the chelating bond, it can be assumed as the first approximation that with increasing ionic character in the metal-oxygen bond, the value of parameter, V_0, decreases and the absorption maximum shifts toward a longer wavelngth. The ionicity is dependent upon the difference of electronegativity between the metal and the oxygen atom. When the chelating bond has a pure covalency, the conjugated system is considered to be similar to that of polyene which has the same number of conjugated double bond, whereas it becomes equal to that of symmetrical polymethine when the chelating bond is pure ionic. The values of V_0 calculated from the experimentally

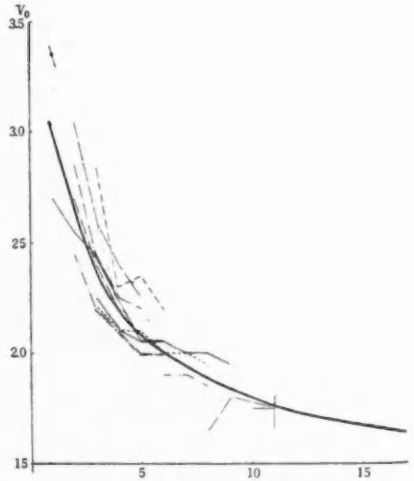


Fig. 3. Correlation between V_0 and Number of Conjugated Double Bond in Polyenes.

* The value of L, 14.6 Å, is reasonable, because this value is comparable to that obtained in the same manner[9] for many cyanine dyes possessing the same number of conjugated double bond. See also the references 8, 9 and 11 with respect to the propriety to apply the one dimensional free electron gas model method to the branched double bond system.

TABLE I

Metal	H	Ge	Sn	Ti*	Zr	ionic
λ_{max} (mμ)	468	510	510	525	540	560
ε_{max}	4.3×10^4	8.5×10^4	1.2×10^4	4.7×10^4	15×10^4	4.8×10^4
Ratio, Reagent: Metal.	(1 : 1)	2 : 1**	—	2 : 1	4 : 1	—
Ionic Radius[13] (Å)	—	0.53	0.71	0.68	0.80	—
Electronegativity[14]	2.1	1.8—1.9	1.8—1.9	1.6	1.5	—
V_0(eV.)	0.49_0	0.23_6	0.23_6	0.16_2	0.094_5	0

* Ti is soluble as a true solution in 0.1 N or more concentrated hydrochloric acid.
** This value was gven by Dr. K. Saito.

determined absorption maxima of a number of polynes* have been collected in Fig. 3, showing the relationship between V_0 and number of conjugated double bond.

By the aid of the Pauling's[14] or Hannay-Smyth's empirical equation[15] or Dailey-Townes' empirical diagram[16], the ionic character of the chelating bond between the metal and the oxygen atom was estimated from the electronegativity of the atoms concerned**. The relationship between V_0 and ionic character I (or covalent character, C) will be expressed by the following equation,

$$V_0=F(1-I), \text{ or } V_0=F(C). \qquad (3)$$

In this equation, the function F should have the property that V_0 increases up to the value of corresponding polyene with decreasing I or increasing C, whereas it becomes zero when I reaches unity or C comes to zero. The following n'th power function, one of the functions expected, is applied to examine the experimental results;

$$V_0=k(1-I)^n \quad \text{or} \quad V_0=k\cdot C^n \qquad (4)$$

where k and n are parameters to be determined by the experiment. The values of V_0 calculated from the experiments for the metal-phenylfluorone chelates are listed in Table I, together with other characters of the metal concerned. The value of n was determined from the relationship between the value of V_0 and the covalent character of the bond. The value of k is equal to that of V_0 obtained for the polyene which has the same number of conjugated double bond as phenylfluorone. These results were shown in Fig. 4a, b and c, in which all data met a straight line relation.

These results show that the shift of the wavelength of absorption maximum in the metal chelate depends on the ionic character of the bond between the metal and the functional atom of reagent. Conversely this fact makes it possible to estimate qualitatively the ionic character of the bond or the electronegativity of the metal concerned.

For many oragnic dyes used for the colorimetric determination of various metals, it is generally known that when the dye reacts with metal the absorption maximum shifts toward a longer wavelength. These phenomena will be explained as follows.

From equation (1), the following equation is derived,

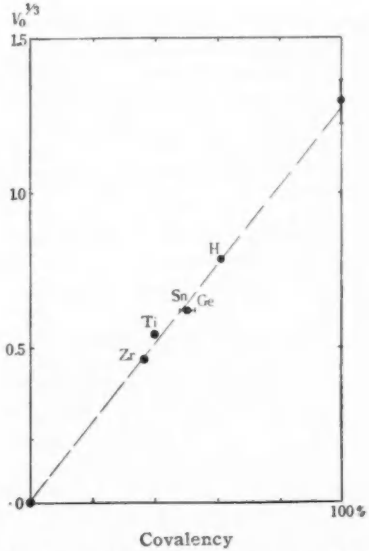


Fig. 4, a. Based on Pauling's equation.

* The absorption maxima were taken from the following: A. E. Gillam and E. S. Stern "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry", Edward Arnold Ltd. London, (1954); Y. Urushibara, "Organic Chemistry II", Kyoritsu Shuppan, Tokyo, (1955).

** The value of electronegativity was taken from the Table presented recently by H. O. Pritchard and H. A. Skinner, *Chem. Rev.*, **55**, 745 (1955).

14) L. Pauling, "The Nature of the Chemical Bond", Cornell Univ. Press, Ithaca, 1939.

15) N. B. Hannay and C. P. Smyth, *J. Am. Chem. Soc.*, **68**, 171 (1946).

16) B. P. Dailey and C. H. Townes, *J. Chem. Phys.*, **23**, 118 (1955).

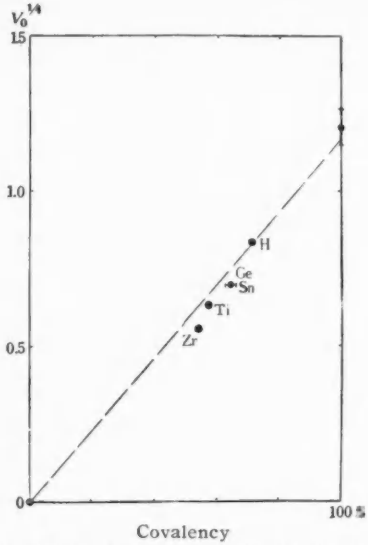


Fig. 4, b. Based on Smyth-Hanney's equation.

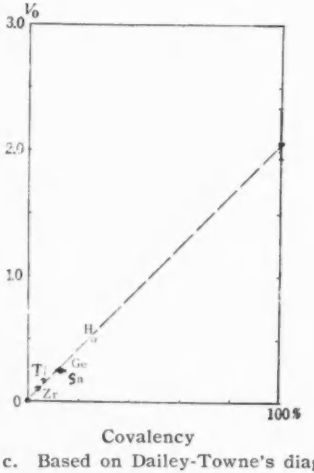


Fig. 4, c. Based on Dailey-Towne's diagram.

$$V_0=N\cdot h(\nu-\nu_i)/(N-1)=N\cdot h\{(\nu_H-\nu_i)-(\nu_H-\nu)\}/(N-1)$$
$$=N\cdot h(\nu_0-\Delta\nu)/(N-1) \qquad (5)$$

where ν is the frequency of absorption maximum observed with the metal chelate, ν_H and ν_i, the frequency of absorption maximum of the reagent in the acidic and the alkaline solution, respectively, and $\Delta\nu$, the shift ("red shift") of the frequency, which is equal to $(\nu_H-\nu)$. From the equation (4) and (5), $\Delta\nu$ can be derived as follows,

$$\Delta\nu=\nu_0-k(N-1)(1-I)^n/N\cdot h=\nu_0-k(N-1)\cdot C^n N\cdot h \qquad (6)$$

where $\nu_0(=\nu_H-\nu_i)$ is constant for the reagent used. From equation (6), it is seen, that $\Delta\nu$ is affected with the covalent character of the bond, where k and n are chracteristic of the reagent used. In general, however, this relation will not always be satisfied because of their unsymmetrical structure, branched conjugated system, or uncertainty of the ionicity of the bond which is estimated empirically from the difference of electronegativity.

Summary

The absorption spectra of phenylfluorone and its chelates with the fourth group metal elements, titanium, germanium, zirconium and tin were studied. It was found that in acidic solutions the absorption maxima of these metal chelates shift towards a longer wavelength with increasing ionicity of the bond between the metal and the terminal oxygen atom of the ligand phenylfluorone. These absorption spectra were examined by the aid of free electron gas model method. Kuhn's theory was applied to the calculation of the value V_0 from the absorption maximum observed in each metal chelate. It was shown that there was a certain relationship between the value of V_0 obtained and the ionicity of the chelating bond which is estimated from the difference of electronegativity between the metal and the terminal oxygen atom. The relation can be expressed as follows:

$$V_0=k(1-I)^n \qquad (4')$$

where, n is a parameter depending on the method used for the estimation of the ionicity, k a parameter characteristic of the chelating dye used and equal to the value of V_0 for the polyene having the same number of the conjugated double bond. These results were applied to explain the "red shift" observed in acidic solutions of organic dyes in the colorimetric determinations of metals. A more detailed investigation concerning the nature of the chelating bond will be reported later.

The author indebted to Dr. Kenjiro Kimura, former Professor and Dr. Nobufusa Saito, Professor in this laboratory, for their benevolent encouragement to carry out this study.

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Studies on Crystals of Metallic Tris-ethylenediamine Complexes. III. The Determination of the Absolute Configuration of Optically Active Complex Ion, [Co en$_3$]$^{3+}$, by Means of X-rays

By Yoshihiko SAITO, Kazumi NAKATSU, Motoo SHIRO* and Hisao KUROYA

(Received May 23, 1957)

Introduction

In 1951, Bijvoet, Peerdeman and van Bommel[1] first succeeded in determining the absolute configuration of *d*-tartaric acid by means of X-rays, using absorption edge technique.

However, no attempt has ever been made to determine the absolute configuration of optically active tris-ethylenediamine-cobaltic complex ion, [Co en$_3$]$^{3+}$ (en: ethylenediamine), one of the most familiar complex ions in the chemistry of complex compounds. The present paper deals with this problem, including a brief description of the principle of the method and the experimental procedure. A brief account of this work has been published already[2].

Principle of the Method

As is well known, Friedel's law states that it is not possible, by ordinary methods in X-ray diffraction study, to detect the presence or the absence of centrosymmetry in a crystal, from which it follows immediately that in the case of non-centrosymmetry one can not conclude whether a model or its inversion actually corresponds with a given optically active crystal, say—dextro compound. However, it is not true when use is made of X-rays whose wave length is a little shorter than the absorption edge of a particular atom in the crystal. If this condition is fulfilled, a difference in intensity between the reflections from (hkl) and $(\bar{h}\bar{k}\bar{l})$ can be observed for non-centrosymmetrical crystal.

This is due to the scattering of X-rays by the particular atom with anomalous phase and amplitude. From the difference between $I(hkl)$ and $I(\bar{h}\bar{k}\bar{l})$, one can determine the absolute configuration of a structure which belongs to one of the pairs of enantiomorphous space groups, provided that the crystal structure is fully known in all respects except that of "hand". The actual intensities $I(hkl)$ and $I(\bar{h}\bar{k}\bar{l})$ can be calculated on the basis of a final set of atomic coordinates determined in the usual way, taking the anomalous dispersion into account. If the observed inequality relations are the reverse of those calculated, the real atomic parameters must be the inversion of the initial set.

Besides these inequality relations, the following equality exists:

$$I_d(hkl)=I_l(\bar{h}\bar{k}\bar{l}) \qquad (1)$$

where the subscript d and l are used to distinguish the two enantiomorphous crystals. It follows immediately:

$$\text{if } I_d(hkl)\gtrless I_d(\bar{h}\bar{k}\bar{l}), \text{ then } I_l(hkl)\gtrless I_l(\bar{h}\bar{k}\bar{l}), \qquad (2)$$

that is, the inequality relations must be reversed in the case of *d*-compound and *l*-compound.

Since the wave length of Cu $K\alpha$ radiation (λ=1.5418 Å) is a little shorter than the *K*-absorption edge of the cobalt atom (λ_k=1.6081 Å), anomalous dispersion is expected to take place. The crystal structures of D- and L-2[Co en$_3$]Cl$_3$·NaCl·6H$_2$O are fully known except that of "hand" as reported previously[3]. Therefore, the absolute configuration of these compounds can be determined without ambiguity, if the difference between $I(hkil)$ and $I(\bar{h}\bar{k}\bar{i}\bar{l})$ can be observed when Cu $K\alpha$ radiation is used.

The phase change on scattering may be included in the usual expression for the structure factor without changing its form, if we consider the atomic scattering factor to be complex numbers[4]:

$$f=f^0+\Delta f'+i\Delta f'' \qquad (3)$$

* Present adress; Momotani Juntenkan Co. Ltd., Osaka.

1) J. M. Bijvoet, A. F. Peerdeman and A. J. van Bommel, *Nature*, **168**, 271 (1951).

2) Y. Saito, K. Nakatsu, M. Shiro and H. Kuroya, *Acta Cryst.*, **8**, 729 (1955).

3) K. Nakatsu, M. Shiro, Y. Saito and H. Kuroya, This Bulletin, **30**, 158 (1957).

4) R. W. James, "The Optical Principles of the Diffraction of X-rays", G. Bell and Sons, Ltd., London (1948), Chap. IV.

where f^0 is the scattering factor for wave lengths far from any one of the absorption edges of electrons and $\Delta f'$ and $\Delta f''$ represent the corrections to be applied to the real and the imaginary part of the scattering factor because of dispersion.

When Cu $K\alpha$ radiation is used to take diffraction photographs of the complex compound above mentioned, the K-level electrons of the cobalt atom are excited. Therefore, we have for the scattering factor of the cobalt atom:

$$f_{Co}=f^0_{Co}+\Delta f'_{Co}+i\Delta f''_{Co} \qquad (4)$$

The structure factor $F(hkil)$ for $l=2n$ can be written as follows, if we assume regular structure postulated in the previous paper:

$$F(hkil)=A(hkil)+iB(hkil), \qquad (5)$$

where

$$A(hkil)=\sum_j m_j f_j \cos 2\pi l z_j \{\cos 2\pi(hx_j+ky_j)+\cos 2\pi(kx_j+iy_j)+\cos 2\pi(ix_j+hy_j)\}$$

$$B(hkil)=\sum_j m_j f_j \sin 2\pi l z_j \{\cos 2\pi(hx_j+ky_j)+\cos 2\pi(kx_j+iy_j)+\cos 2\pi(ix_j+hy_j)\}$$

and

f_j: atomic scattering factor for the j-th atom

m_j: a suitable factor to allow for the reduced multiplicity for atoms on the special positions

x_j, y_j, z_j: atomic coordinates for the j-th atom.

It can easily be shown from the above expression that $F(hki\bar{l})$ is equivalent to $F(\bar{h}\bar{k}il)$ and is a complex conjugate of $F(hkil)$. Because of eq. (4), $|F(hkil)|$ is no more equal to $|F(hki\bar{l})|$, hence, the intensity $I(hkil)$ is unequal to $I(hki\bar{l})$. Accordingly, to observe the effect of anomalous dispersion, it is more convenient to compare the reflections from $(hkil)$ and $(hki\bar{l})$ instead of those from $(hkil)$ and $(\bar{h}\bar{k}il)$. These reflections can be observed on the same oscillation photograph taken around the c-axis and moreover, various correction factors come out exactly the same for these two reflections.

Experimental Procedure and the Determination of the Absolute Configuration

i) *Observation of Inequality Relations by Oscillation Photographs.*

Thin needle-like crystals of about 0.2 mm. in diameter were selected. First, a few oscillation photographs were taken using Fe $K\alpha$ radiation, in order to confirm that the crystal setting is perfect and $(hkil)$ and $(hki\bar{l})$ intensities are practically equal. Photographs were then taken with Cu $K\alpha$ radiation, which is scattered by the cobalt atoms anomalously. In Fig. 1, the effect of anomalous dispersion is shown, where reflections of Fe $K\alpha$ and Cu $K\alpha$ from the plane $(32\bar{5}2)$ and $(325\bar{2})$ are recorded on one film, the latter being equivalent to $(3\bar{2}5\bar{2})$ because of symmetry. For D-compound the intensity of reflection of Cu $K\alpha$ from the plane $(325\bar{2})$ is clearly stronger than that from the plane $(32\bar{5}2)$, while they are equal in intensity in case of Fe $K\alpha$ radiation. The inequality relation is reversed for L-compound.

There are many such pairs of reflections for which slight but discernible differences in intensities were observed, whereas no such differences could be found when Fe $K\alpha$ radiation was used.

It was also found that the intensity relations observed for the one crystal are the reverse of those found for its enantiomorph. This observation strongly suggests that these differences are due to the anomalous dispersion of cobalt atoms in the crystal. In Table I observed and calculated relations between $I(hkil)$ and $I(hki\bar{l})$ are compared for all the observed pairs. The visual estimates of the last two columns are the results of observations by a few persons independently. In Table I the inequality sign $>$ or $<$ shows that the observed intensity for $(hkil)$ is greater or smaller than that for $(hki\bar{l})$. The sign $?>$ or $?<$ means that the inequality is not so definite as that without the symbol ?. The symbol ? shows that the inequality could not be observed or was different according to different observers. In the calculation of intensities of reflections, $\Delta f'$ and $\Delta f''$ were calculated according to the formula derived by Hönl[5]. They amount to $\Delta f'=-3.0$ and $\Delta f''=3.4$, respectively. The calculated values shown in the first column are based on the regular model. Those shown in the second column were calculated on the basis of a model having disorder due to submicroscopic twinning. In this case, $F(hki2n)$ is not exactly equal but very near to the complex conjugate of $F(hk\bar{i}2n)$ even when no anomalous dispersion occurs. Thus the difference between $I_c(hkil)$ and $I_c(hki\bar{l})$ calculated for Fe $K\alpha$ radiation is

5) H. Hönl, *Zeit. f. Physik*, **84**, 1 (1933); *Ann. Physik*, **18**, 625 (1933).

TABLE I

OBSERVED AND CALCULATED INTENSITIES

	Calculated on the Basis of				Observed for	
	Regular Model		Twinned Model			
hkil	$I(hkil)$	$I(hki\bar{l})$	$I(hkil)$	$I(hki\bar{l})$	D-Cryst.	L-Cryst.
1012	1985	1810	2300	2140	?	<
1122	2.9	159	17	100	<	>
1232	86	173	196	381	<	>
1342	965	910	921	865	? >	? <
1452	781	705	824	738	? >	? <
1562	312	296	300	292	>	<
1892	51	47	50	47	? >	<
1, 9, 10, 2	33	34	33	37	—	? >
2022	148	269	334	423	<	>
2132	3710	3460	3310	3200	>	<
2242	38	159	30	102	<	>
2352	418	448	444	485	? <	? >
2462	112	99	124	112	?	?
2572	15	17	17	14	?	?
2682	6.4	7.1	6.8	11.5	—	>
2792	71	65	67	62	? >	?
2, 8, 10, 2	84	75	101	89	? >	?
2, 9, 11, 2	47	41	45	41	? <	—
3032	72	167	146	171	<	>
3142	315	434	514	557	<	>
3252	141	82	120	82	>	<
3362	165	194	212	241	? <	? >
3472	34	46	25	35	? <	?
3582	146	141	149	146	? >	? <
3, 7, 10, 2	58	61	59	68	? <	?
3, 8, 11, 2	39	32	35	29	? >	?
4042	1252	1160	1106	1083	? >	? <
4152	21	27	45	25	<	>
4262	291	310	310	329	?	?
4482	3.3	28	2.2	24	<	>
4, 7, 11, 2	0.4	6.9	2.1	4.1	<	>
5052	412	394	371	363	?	?
5272	0.7	11	3.3	6.7	<	>
5382	86	76	84	77	? >	<
5492	99	104	105	115	? <	? >
6172	43	37	41.3	41.1	>	<
6282	88	86	84	86	? <	>
6392	187	169	207	178	? >	?
6, 4, 10, 2	125	112	112	106	? >	<
7072	233	213	219	196	?	?
7292	164	131	123	120	>	<
7, 3, 10, 2	93	94	92	94	?	? <
7, 4, 11, 2	0.1	6.2	2.7	4.8	? >	?
8192	5.1	7.9	6.0	10.4	—	>
8, 2, 10, 2	9.4	4.9	13.4	5.2	>	<
8, 3, 11, 2	5.2	5.4	5.2	6.9	?	—
9, 1, 10, 2	37	40	40	44	?	?
10, 0, 10, 2	14	12	12	9.8	? >	—

small and within the errors of experiments for most cases, and the calculated intensities based on the latter model for Cu $K\alpha$ radiation lead to the same inqeuality relations with only a few exceptions. Therefore, the same conclusion was drawn from both models.

Namely, the concordance, in general, of intensity signs in the observed and the calculated intensities seen in the Table I indicates that the drawing of a complex ion $[Co\ en_3]^{3+}$ shown in Fig. 2 corresponds to the dextrorotatory form with respect to Na D line. This result has been indeed verified by computing the $P_s(\mathbf{u})$ function of Okaya, Saito and Pepinsky[6,7].

ii) *Observation of Inequality Relations by Laue Photographs.*

Grenville-Wells and Lonsdale[8] showed that the effect of anomalous dispersion could also be observed by means of Laue photographs. This method has certainly some advantages over the one using oscillation photographs. However, when applied to the present case, the observation could not be carried out without experimental difficulties. Since the crystals disintegrated by losing their water of crystallization when exposed in the X-ray beam for several hours, it was difficult to set the crystal perfectly and obtain appropriate Laue photographs before decomposition. Moreover, no suitable pairs of reflections could be found which were of high order and strong enough in intensity to reduce the influence of the setting error of the crystal.

Though not satisfactorily, the inequality relations were observed about a few pairs of (*hki*2) reflections in accord with the results obtained by using oscillation photographs.

Some Remarks on Experimental Procedure

It is to be noted here that the same coordinate system must be used in the indexing of the oscillation photographs and calculation of the structure factors. The right handed system was used throughout this investigation. As pointed out by Bijvoet and his coworkers, the calculation of the differences between (hkl) and $(\bar{h}\bar{k}\bar{l})$ intensities does not need very accurate F-values. Theoretically, observation of a single pair of reflections is enough to decide the diffraction model and its mirror image. However, it is advisable to observe the difference of as many corresponding spots as possible. From this point, the Laue photographic method might be laborious.

When anomalous dispersion occurs in the crystal, incident beam is in general heavily absorbed and fluorescent radiation is copiously excited, so that the photograph shows weak diffraction spots on a rather heavily blackened background. Usually its takes a pretty long time to take a diffraction photograph. To avoid the blackening as much as possible and to obtain a picture with clear background in a relatively short time, it is necessary to make the range of oscillation of the crystal small.

Practically it is a rather difficult task to compare the intensities from a front (hkl) and a rear face $(\bar{h}\bar{k}\bar{l})$ of a crystal, since it is not easy to obtain a crystal whose front and rear surface are exactly at the same condition with regard to their perfection etc. However, in most cases it is possible to avoid this difficulty. If, for instance, $F(hkl)$ is equivalent to $F(hk\bar{l})$ because of the symmetry of the space group, it is more convenient to use the reflection $(hk\bar{l})$ instead of $(\bar{h}\bar{k}\bar{l})$, as pointed out in the previous section.

The best method to measure the difference of $I(hkl)$ and $I(\bar{h}\bar{k}\bar{l})$ would be to use two Geiger counters arranged in such a way that the two reflections are received at the same time and to use a circuit by which the difference can be read off directly.

Summary

The absolute configuration of an optically active complex ion $[Co\ en_3]^{3+}$ has been determined by means of X-ray absorption edge technique. Cu $K\alpha$ radiation was used to excite the cobalt atoms in the crystals of D- and L-2$[Co\ en_3]Cl_3 \cdot NaCl \cdot 6H_2O$. The effects of the anomalous dispersion were observed by single crystal oscillation photographs as well as Laue photographs. A brief description of the principle of the method and the experimental procedure is included.

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6) Y. Okaya, Y. Saito and R. Pepinsky, *Phys. Rev.*, **98**, 1857 (1955).
7) Y. Saito, Y. Okaya and R. Pepinsky, In press; cf. also, Phys. Rev., **100**, 970 (1955).
8) H. J. Grenville-Wells and K. Lonsdale, *Nature*, **173**, 1145 (1954).

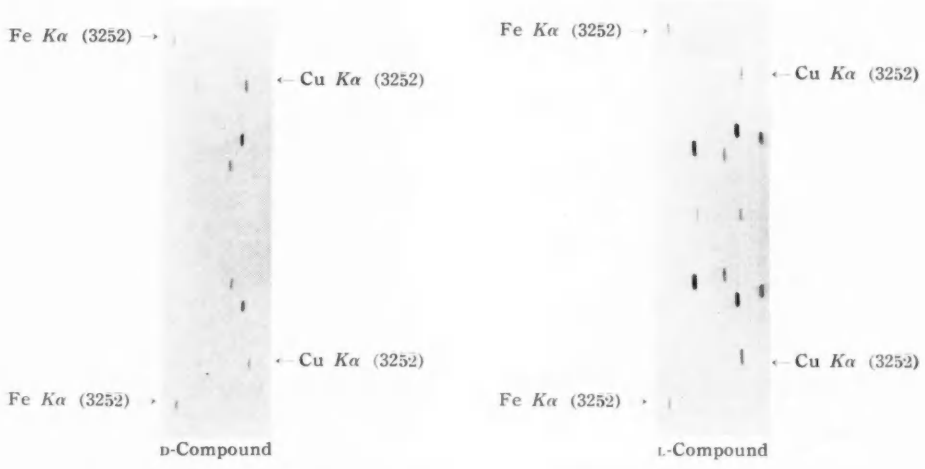

Fig. 1. Parts of oscillation photographs of D- and L-2[Co en_3]Cl_3·NaCl·$6H_2O$, showing the effect of anomalous dispersion.

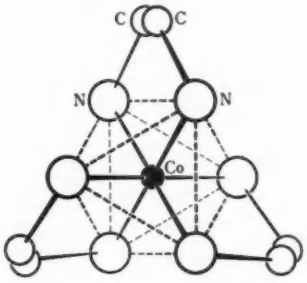

Fig. 2. Drawing of the D(+)-[Co en_3]$^{3+}$ complex ion.

A Rapid Spectrophotometric Method for Determination of a Minute Amount of Vanadium in Natural Waters

By the late Hideo NAITÔ and Ken SUGAWARA

(Received April 15, 1957)

In a previous paper[1] the writers reported with M. Tanaka a method for determining a minute quantity of vanadium in natural waters, consisting of the following three steps: 1) co-precipitation of vanadium with ferric hydroxide, 2) separation of the vanadium from the co-precipitant and from the accompanying elements, and 3) the final colorimetry of vanadium oxinate in pentanol.

The new method which we will detail below is an improvement on this previous method. The essential improvement is in simplifying the processes for vanadium separation from co-precipitant. The simplification is based upon the findings that so long as the solution is sufficiently cool below room temperature, tartrate can keep iron from hydroxide formation even in a strongly alkaline medium and that when the solution is warmed in this condition, iron and aluminium can easily be precipitated leaving vanadium completely in solution.

Experimental

Reagents.—*Mixed solution of sodium hydroxide and tartaric acid.*—Dissolve 30 g. of tartaric acid and 200 g. of sodium hydroxide in distilled water and dilute to 1000 ml.

Ferric chloride solution.—Dissolve 24.2 g. of ferric chloride crystal (the special grade for analytical purposes) in distilled water. Add 5 ml. of 6 N hydrochloric acid and dilute to 500 ml.

Sodium acetate buffer solution.—Dissolve 136 g. of sodium acetate in about 200 ml. of distilled water. Heat the solution on a water bath, then filter through a filterpaper. To the filtrate add 3 ml. of glacial acetic acid and dilute to 250 ml.

Oxine solution.—To 100 ml. of distilled water, dissolve 2 g. of oxine (special grade for analytical purposes) and 5 g. of malonic acid crystal.

Procedure.—Take a sample of 5—20 liters depending upon the probable content of vanadium. Acidify with hydrochloric acid. Then add a solution of ferric chloride, ferric nitrate or iron alum so that the solution contains 10 mg. iron per liter. While warming in a water bath, the solution is neutralized with 4 N aqueous ammonia to bromcresolpurple and the precipitate is collected by an automatic filter.

Gently burn to ashes the filterpaper with its contents in a platinum dish. To the ash add 5 ml. of 6 N hydrochloric acid. Then heat the dish with a cover on a steam bath. Loss of hydrochloric acid causes no essential trouble. The final volume of the hydrochloric acid must not exceed 5 ml. After complete dissolution, transfer the contents to a 100 ml. beaker. Wash the dish with 50 ml. of distilled water. With constant stirring, add 15 ml. of the tartrate-sodium hydroxide solution. The solution turns dark brown without precipitation.

Heat the solution on a steam bath until ferric hydroxide completely precipitates and the supernatant solution is left clear and colorless. The process usually takes 30 minutes and sometimes longer. After it has cooled to room temperature, dilute the solution exactly 100 ml. and put to centrifugation for 10 minutes at 3000 r.p.m.

Transfer 80 ml. of the supernatant solution to a 100 ml. beaker, neuturalize to *p*-nitrophenol with 4 N sulfuric acid. Then add 3 ml. of the same solution in excess. Heat in a water bath. To the hot solution add 2 ml. of the oxine solution, neutralize with 4 N ammonia and then add 4 ml. of the same reagent in excess, whereby a trace of iron which escaped from the previous precipitation process is fixed in the form of oxinate, partly precipitated and partly in solution. When the precipitate is appreciably great, it can be separated by centrifugation. Otherwise go directly on to the next process.

The next process is the extraction of the formed oxinate by an organic solvent. Transfer the solution into a separatory funnel and add about 20 ml. of benzene. Shake vigorously for one minute. Two benzene extractions are sufficient for complete removal of the iron oxinate.

Neutralize the remaining solution with 4 N sulfuric acid and add 3.0 ml. of the same acid in excess. Adjust the pH to nearly 4 by the addition of 4 ml. of a sodium acetate solution. Then add 1.0 ml. of an oxine solution and extract the formed vanadium oxinate by pentanol. The extraction is repeated until the final extract bears no perceptible coloration. Transfer the united extracts into a 10 ml. measuring flask and make up with pentanol to the mark. Then the optical density of the solution is determined by using a spectrophotometer at wave length of 475 mμ.

1) K. Sugawara, M. Tanaka, and H. Naitô, This Bulletin **26**, 417 (1953).

Discussion

Whether the vanadium co-precipitation with ferric hydroxide is complete or not depends upon the pH of the milieu. As shown in Table I[2], it is complete only

TABLE I
VANADIUM CO-PRECIPITATION BY IRON AT VARIOUS pH VALUES

pH of the solution	Fe added mg.	V added μg.	V found in the ppt. μg.
7.9	2	33.0	33.2
8.8	2	33.0	32.2
9.6	2	33.0	33.2
10.8	2	33.0	<1
12.3	2	33.0	<1

within the pH range up to 9.6. Therefore, in the first step of the precedure the precipitation was arranged so that it occurs at that pH whereby a trace of vanadium can be completely collected in the coagulum.

The situation is quite contrary for the second step of the procedure where vanadium must remain in solution to its trace when hydroxides are precipitated. The requirement is that:

1) In the alkalinization process, iron and aluminium must be kept in solution until the pH of the solution becomes sufficiently high for these elements to precipitate free from a trace of vanadium.

2) Finding a process which causes a homogeneous precipitation at this pH of the iron and aluminium, which have been kept in solution up to the pH.

The addition of tartrate satisfies this requirement. At the stated pH, iron and aluminium can be masked by this substance at low temperatures and easily demasked with precipitation when warmed. Recovery of vanadium was the final question which was solved by the following experiment:

To a series of solutions containing various amounts of vanadium, 100 ml. of iron and 500 ml. of a tartaric acid solution were added. Each solution was made alkaline by the addition of 15 ml. of 5 N sodium hydroxide solution which had previously been cooled below room temperature. Then the solution was warmed in a boiling water bath whereby iron hydroxide was precipitated. The supernatant solution thus obtained was processed as given in the procedure. As shown in Table II, the recovery of vanadium is perfect.

TABLE II
RECOVERY OF VANADIUM FROM IRON PRECIPITATE

Fe added mg.	V added μg.	V found μg.	%
100	20	19.5	98
100	20	19.6	98
100	20	20.4	102
100	20	20.8	104
100	20	19.6	98
100	20	19.7	99
100	20	19.9	100
100	8	8.1	101
100	8	8.5	106

As to the amount of iron added, when it is less than 5 mg., separation is complete without the addition of tartrate, and when it exceeds 20 mg., hydrogen peroxide treatment is not sufficient for a complete separation, which can be first realized by the addition of tartrate.

Interferences by other elements.

Some other elements remaining in the solution from which ferric hydroxide had been separated, interfere with the final colorimetry of vanadium by forming oxinates. By the addition of malonic acid, aluminium can be prevented from forming oxinate in a neutral or a slightly acidic solution. No harmful effect is caused by the presence of 50 mg. of aluminium, 10 mg. of manganese or zinc, and 1 mg. of cadmium, bismuth, copper, silver, mercury, tin, selenium, tungsten, molybdenum or titanium.

The present study was accomplished by using a Coleman Model 14 Universal Spectrophotometer supplied by the Elizabeth Thompson Science Fund. The writers express their appreciation to the Trustees of that Fund.

The senior writer is sorry to report the tragic death on October 11, 1956, of his co-worker, H. Naitô, when during his surveying work on Lake Kizaki-ko, rough water suddenly capsized the boat and was drowned.

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2) M. Tanaka, unpublished.

*Sedimentation and Diffusion Studies of Calf Thymus Histone**

By Nobuo Ui

(Received April 30, 1957)

In a previous paper[1], it was reported that calf thymus histone consists of two fractions, histones I and II, which were different in respect to chemical as well as physico-chemical properties. The present paper deals with the molecular-kinetic properties of these two fractions. In spite of their heterogeneity as revealed by electrophoresis, they behaved like a single protein in sedimentation and diffusion, and the molecular weights have been calculated.

As there have been few data about the molecular weight of histone, especially of mono-disperse preparation, it seems of value to present the results in some detail. A preliminary report of this investigation has already been published[2].

Experimental

Materials.—The preparations of histone used in this study were extracted from isolated calf thymus nuclei using sulfuric acid, purified by an ethanol-precipitation method at a temperature below 0°C, and finally lyophilized as histone chlorides. Histone I is the main fraction of histone precipitated with 20 per cent. ethanol, and histone II is the fraction precipitated by 45 per cent. Details of the procedure for the preparation and fractionation have been described[1].

As it was found that histone I had a tendency to aggregate, especially at higher temperature, care was taken to avoid the aggregation; i.e., each preparation was dissolved in a cold buffer solution and kept at about 0°C until measurements were performed (less than one day). In most of this study an acetate buffer of pH 5.0 at ionic strength of 0.2 was used as a solvent. In this the aggregation did not take place appreciably even if the solution was kept at 20°C for 24 hours.

Protein concentrations were usually determined by the micro-Kjeldahl method. The author is indebted to Miss T. Kitamura for this analysis.

Methods.—Ultracentrifugal characteristics of these materials have been studied using a Spinco model E ultracentrifuge. In the case of histone II, a synthetic boundary cell[3] was used in order to measure its low sedimentation coefficient. Runs were made always at 59,780 r. p. m. The temperature of each run was taken as the mean of the rotor temperature at the beginning and the end of the run without correction for a change in rotor temperature during acceleration and deceleration[4]. The observed sedimentation coefficient was corrected to the value in water at 20°C ($s_{20,w}$) by a conventional manner[5].

Diffusion measurements were made at 20.0°C in a Neurath-type diffusion cell[6] equipped with a Philpot-Svensson's schlieren optical system[7]. Diffusion coefficients were computed from the second moments of the diffusion curves[8] and corrected to the value in water at 20°C ($D_{20,w}$).

Partial specific volume of histone I was determined at 20.0°C, using a pycnometer of 5-cc. capacity.

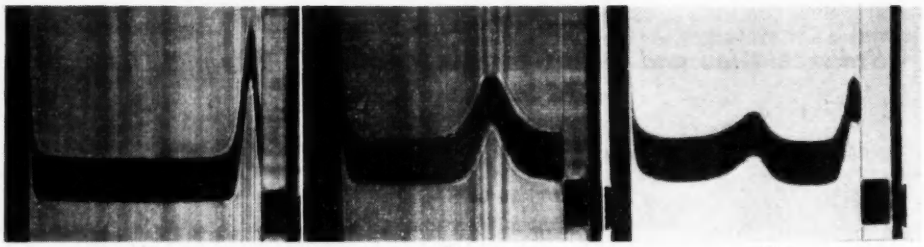
Results

A. Sedimentation Characteristics of Histone I.—The preparation of histone I, the main fraction of calf thymus histone, was examined in the ultracentrifuge in a pH range of between 4.0 and 9.9. Only a single homogeneous boundary was observed below pH 7, as is shown in Fig. 1 (1A and 1B). These photographs were taken using an acetate buffer of pH 5.0 and ionic strength of 0.2.

At pH values higher than 7, except in tris(hydroxymethyl)aminomethane buffer of pH 8.0, a faster component was observed (see Fig. 1C) even when the solution had not been exposed to a high temperature. The amount of the fast component varied with the pH and the composition of the buffer. It was also found that the faster boundary disappeared when the solution was quickly brought to pH 5.0 by dialysis in a cold room and examined by the ultracentrifuge. The presence of the fast component at a higher pH than 7 would not be due to the inhomogeneity of this preparation, but would

* A part of this work was read before the 8th Annual Meeting of the Chemical Society of Japan held in Tokyo, April 1, 1955.

1) N. Ui, *Biochim. Biophys. Acta*, in press.
2) N. Ui, *Biochim. Biophys. Acta*, **22**, 205 (1956).
3) H. K. Schachman, *Proc. Natl. Acad. Sci. U. S. A.*, **38**, 943 (1952).
4) D. F. Waugh and D. A. Yphantis, *Rev. Sci. Instruments*, **23**, 609 (1952).
5) T. Svedberg and K. O. Pedersen, "Ultracentrifuge," Oxford (1940).
6) H. Neurath, *Chem. Revs.*, **30**, 357 (1942).
7) J. St. L. Philpot, *Nature*, **141**, 283 (1938); H. Svensson. *Kolloid-Z.*, **87**, 181 (1939).
8) O. Lamm, *Nova Acta Regiae Soc. Sci. Upsaliensis*, Ser. IV, **10**, No. 6 (1937).



(A) (B) (C)

Fig. 1. Sedimentation patterns of calf thymus histone I.
(A): In acetate buffer of pH 5.0 at ionic strength of 0.2 after 23 min. at 59,780 r. p. m.
(B): The same as (A), but after 190 min.
(C): In veronal-NaCl buffer of pH 9.0 at ionic strength of 0.2, after 16 min. at 59,780 r. p. m.

be accounted for if a (reversible) formation of aggregate took place in an alkaline solution.

In Fig. 2 (curve I) is shown the concentration dependency of the sedimentation coefficient of histone I in the acetate buffer of pH 5.0 at ionic strength of 0.2. The sedimentation coefficient slightly increased with decrease in concentration. A value of 2.0_3 S (Svedberg unit) was obtained when extrapolated to infinite dilution by the least square method.

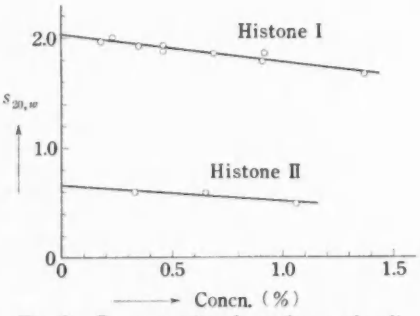


Fig. 2. Concentration dependency of sedimentation coefficients of histones I and II in an acetate buffer of pH 5.0 at ionic strength of 0.2.

The sedimentation coefficients obtained at other pH values are given in Table I. In all cases, the concentration was nearly 1.0 per cent. Amounts of the faster component, if present, are also recorded in the last column. It was noticed that a little higher sedimentation coefficient was obtained at a higher pH.

B. Diffusion of Histone I.—The measurements of free diffusion of histone I were made at 20.0°C in the acetate buffer of pH 5.0 at ionic strength of 0.2. Runs lasted less than 20 hours. Therefore, the effect of aggregation would be small.

Diffusion curves obtained were always symmetrical and showed nearly Gaussian curves. The homogeneity of this preparation was thus confirmed. The diffusion coefficients calculated from the second moments of the refractive-index gradient

TABLE I
SEDIMENTATION COEFFICIENT OF HISTONE I IN BUFFER SOLUTIONS OF IONIC STRENGTH 0.2 (Concn. = ca. 1%)

Buffer	pH	Sedimentation coefficient		Amount of fast comp.
		Slow comp. (S)	Fast comp. (S)	(%)
Acetate	4.0	1.5_9	—	0
〃	4.2_5	1.3_6	—	0
〃	5.0	1.7_8	—	0
Acetate-Na_2SO_4	〃	1.5_5	—	trace
Cacodylate-NaCl	6.0	1.7_6	—	0
NaCl (unbuffered)	6.3	1.8_3	—	trace
Cacodylate	6.8	1.8_6	—	0
Phosphate	7.7	2.2_8	19.1	8
Tris*	8.0	2.1_9	—	0
NH_4Cl-NH_3-NaCl	8.6	2.0_5	26.3	32
Veronal-NaCl	9.0	2.1_9	30.2	60
NH_4Cl-NH_3	9.9	2.4_2	22.0	70

* tris(hydroxymethyl)aminomethane buffer.

TABLE II
DIFFUSION DATA ON HISTONE I

Concn. (%)	$D_{20,w} \times 10^7$ (cm^2/sec.)	Y_{max}	X_{max}
0.93	4.9_3	2.11	0.02
0.47	4.9_4	2.01	0.00
0.41	4.8_3	2.00	0.00
0.34	5.3_9	2.03	0.00
0.30	5.5_3	2.03	0.00
0.28	5.1_5	2.13	0.00
Mean	5.1_3	2.05	0.00

curves were almost independent of concentration and the averaged value was taken as the diffusion constant. The results of the experiments are shown in Table II together with the values of Y_{max} and X_{max}, which show the degree of homogeneity and of concentration dependency of diffusion coefficient, respectively*.

C. Partial Specific Volume of Histone I.—The partial specific volume ($\bar{V}$) of histone I was calculated using the equation,

$$\bar{V}=\frac{m_0-(m-h)}{\rho_0 h} \quad (1)$$

where m is the weight of the pycnometer filled with the solution in which h gram of protein is dissolved, and m_0 is the weight of the pycnometer filled with a solvent with density ρ_0.

The calculated values at the concentrations of 0.96_0, 0.64_0 and 0.42_7 per cent. were 0.73_7, 0.73_7 and 0.73_1, respectively. A value of 0.74 was taken as the mean value.

D. The Molecular Weight and Shape of Histone I.—From these results, the molecular weight (M) of histone I was calculated using the formula deduced by Svedberg and Pedersen[9], viz.,

$$M=\frac{RTs}{D(1-\bar{V}\rho)} \quad (2)$$

where R is the gas constant and T is the absolute temperature. The value (*unhydrated* molecular weight), 37,000, was obtained (see Table IV).

When sedimentation and diffusion constants are known, it is possible to calculate the frictional ratio, f/f_0, i.e., the ratio of the frictional coefficient of the protein to that for a compact spherical and unhydrated molecule of the same volume[9]. In the case of histone I, f/f_0 was calculated to be 1.88.

The fairly large value of f/f_0 raised a question concerning the molecular configuration of histone I. If it could be assumed that molecule is a rigid ellipsoid of revolution and the density of the hydrated water has the same value as that of free solvent, its axial ratio could be calculated using Perrin's equation[9]. Since the extent of hydration is not known, the plausible range of values usually accepted, i.e., 0—0.5 gram water per gram protein, was taken. Then the value of axial ratio, 17—11, was obtained for a prolate ellipsoid. However, in view of the assumption involved in this calculation, it is not convincing that histone I is really such an asymmetric molecule. Recently, Scheraga and Mandelkern[10] attempted to interpret observed physical properties of the protein in terms of a rigid "equivalent ellipsoid of revolution" characterized by its effective volume V_e and its axial ratio without any assumption concerning hydration. They showed that the function, β, defined as $Ns[\eta]^{1/3}\eta_i/M^{2/3}(1-\bar{V}\rho)$, is dependent only upon the shape of the hydrodynamic particle. For histone I, β was calculated using the above-mentioned values of sedimentation constant and molecular weight as well as its limiting viscosity number, $[\eta]$, measured in the same buffer, 0.111 (cc./g.). A value of 2.05×10^6 was obtained, which fell outside the range for a rigid ellipsoid ($\beta=2.12\times10^6$ for spheres and $\beta>2.12\times10^6$ for ellipsoids). As the validity of Scheraga and Mandelkern's treatment has not been established, no definite conclusion would be deduced from this consideration*. However, taking the extreme sensitivity of β to errors in experimental data into consideration, the fact that β was not far from 2.12×10^6 might show that histone I is not highly elongated, but is much swollen by imbibition of a solvent**. Recently, Noda[13] has proposed an experimental equation relating the volume fraction concentration and sedimentation coefficient in a suspension of spheres, and, taking advantage of this equation, he explained the large value of frictional ratio in histone I by the increase of apparent volume by solvation. Our finding that the limiting viscosity number of histone I was very sensitive to the change in ionic strength or pH of the solution[11] might support this view.

* For a homogeneous preparation with a single diffusion coefficient independent of concentration, $Y_{max}=2.00$ and $X_{max}=0$[8]. Slightly higher values of Y_{max} than 2.00 in some of the present experiments may be due to the presence of a small amount of aggregate.

9) F. Perrin, *J. phys. radium*, (7) **7**, 1 (1936).

10) H. A. Scheraga and L. Mandelkern, *J. Am. Chem. Soc.*, **75**, 179 (1953).

* Tanford and Buzzell[11] have shown that the value of β for bovine serum albumin calculated by using accurate molecular-kinetic data was definitely lower than the permissible range for rigid ellipsoids.

11) C. Tanford and J. G. Buzzell, *J. Am. Chem. Soc.*, **76**, 3356 (1954).

** Ogston[12] has also considered several methods for making an estimate of dimensions of solute particles from dynamic properties of their solution. According to one of his most reliable methods, IIb, the axial ratio of the spheroidal hydrodynamic model, J, the effective hydrodynamic volume per gram of unhydrated mass, V', and the molecular weight were calculated for histone I from s_0, $d(1/s)/dc$, and $[\eta]$; the values, $J=5$, $V'=1.9$, $M=35{,}000$, were obtained. It was again suggested that histone I is only moderatly elongated, and it carries a fairly large amount of solvent with it.

12) A. G. Ogston, *Trans. Faraday Soc.*, **49**, 1481 (1953).

13) H. Noda, This Bulletin, **30**, 495 (1957).

14) N. Ui, unpublished.

E. Results on Histone II.—Similar measurements were made on histone II, the other fraction of calf thymus histone.

Ultracentrifugal patterns of histone II (see Fig. 3) showed only one boundary

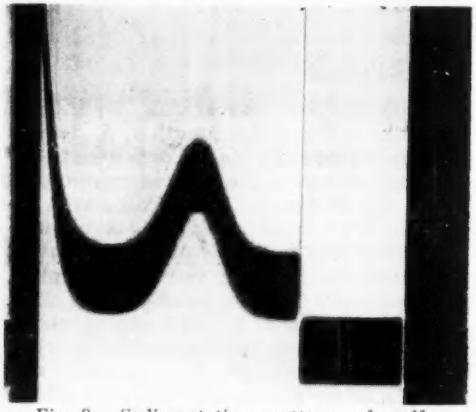


Fig. 3. Sedimentation pattern of calf thymus histone II in acetate buffer of pH 5.0 at ionic strength of 0.2.
After 110 min. at 59,780 r. p. m. in a synthetic boundary cell.

indicating the homogeneity of this preparation. The sedimentation coefficient of this protein was definitely lower than that of histone I in the acetate buffer of pH 5.0 at ionic strength of 0.2. Unlike histone I, histone II did not aggregate even if the solution was kept at 30°C for several days. The sedimentation coefficient vs. concentration curve is given in Fig. 2 (curve II). The sedimentation constant at infinite dilution was obtained by extrapolation as 0.6_6 S.

Diffusion study also confirmed the homogeneity of this preparation. The diffusion coefficients together with the values of Y_{max} and X_{max} calculated from the diffusion curves in the acetate buffer are tabulated in Table III. The mean value was taken as the diffusion constant.

TABLE III
DIFFUSION DATA ON HISTONE II

Concn. (%)	$D_{20,w} \times 10^7$ (cm^2/sec.)	Y_{max}	X_{max}
0.85	7.1_6	2.00	0.00
0.36	7.5_9	2.02	0.00
0.29	7.1_5	2.02	0.01
Mean	7.3_0	2.01	0.00

Assuming the same value of partial specific volume as histone I, the molecular weight of histone II was calculated using equation (2). The value, 8,400, was nearly a quarter of that of histone I. A value of 2.16 was obtained for the frictional ratio, which was even higher than that for histone I.

Several data obtained for histone I and II are listed and compared in Table IV.

TABLE IV
MOLECULAR-KINETIC DATA ON HISTONES I AND II

	Histone I	Histone II
Sedimentation constant, $s_{20,w}$ (S)	2.0_3	0.6_6
Diffusion constant, $D_{20,w} \times 10^7$ (cm^2/sec.)	5.1_3	7.3_0
Partial specific volume	0.74	(0.74)*
Molecular weight	37,000	8,400
Frictional ratio, f/f_0	1.88	2.16
Axial ratio, assuming a rigid ellipsoid without hydration	17	25

* Assumed value.

Discussion

As shown in Table IV, a difference in molecular-kinetic properties is clear between the two fractions of histone, histone I and histone II. The view that histone II is due to the disaggregation of histone I seems unlikely, since histone I could not be converted into a smaller molecule under several conditions examined and the difference of these two fractions was definitely shown by chemical analyses[1]. Therefore, it might be reasonable to assume that calf thymus histone consists of two fractions with different molecular weights; the main fraction (80—90 per cent. of total histone) has a molecular weight of 37,000, and a lower molecular-weight fraction (molecular weight=8,400) is also contained as a minor fraction.

In this connection, it is of interest to compare the results of the present investigation with those reported by other workers. Numerous values of sedimentation constant have been recorded on the preparations of calf thymus histone prepared by various methods, although most other workers used ultracentrifugally inhomogeneous preparations. The presence of a faster component with much higher sedimentation coefficient than 2 S was often reported[15-20], but it is likely that

15) L. Ahlström, *Arki. Kemi, Mineral. Geol.*, **24A**, No. 31 (1947).
16) D. Hamer, *Brit. J. Cancer*, **5**, 130 (1951).

such a component is due to the aggregation that occurred during the isolation or in the solution in which sedimentation was performed. The view was supported by the fact that a similar boundary was also observed in our experiments on histone I at pH values higher than 7 or on using a solution (pH<7) which had been kept at room temperature for a while. The component with a sedimentation coefficient of approximately 2 S was also observed by most investigators[16~20]; the somewhat lower value recorded would be due either to the fact that the values were not extrapolated to infinite dilution or to insufficient separation of a slower component. Although it was reported by some workers that the amount of 2 S component was less than that of the component with a higher sedimentation coefficient[19,20], our conclusion that 2 S component (histone I) is the main fraction of histone would be reasonable, if we consider the great tendency of this component to aggregate. The results of Butler and coworkers aggreed to a fair extent with our results. However, their "slow component" purified as a homogeneous fraction showed higher value of sedimentation constant, 1.06 S[18], than histone II. As the properties and the amino acid composition of these two preparations are similar, further study would be necessary to explain the discrepancy.

The diffusion constant of histone has been measured by Cruft et al.[21], and Butler et al.[17,18] The former authors found that the diffusion coefficient of their preparation (β-histone) depended markedly upon pH and accounted for this phenomenon by aggregation. The highest value reported by them, 4.2×10^{-7} cm^2/sec., which was obtained at pH 1.7, nearly coincided with our values for histone I at pH 5.0. The value obtained by Butler et al. markedly varied with preparations, and it was noticed that even an ultracentrifugally inhomogeneous preparation showed nearly a Gaussian curve[17]. The diffusion constant of a homogeneous fraction with a low molecular weight[18] was 5.3×10^{-7} cm^2/sec., definitely lower than our value for histone II. Luck et al.[20] noted that the diffusion curves of their two fractions were not Gaussian.

Molecular weight of calf thymus histone has been calculated by Butler et al. by combining the values of sedimentation and diffusion constants[17,18]. For the homogeneous fraction with a low sedimentation constant (the slow component) a value of 18,000 was obtained, which was approximately twice as great as that of our histone II. The molecular weights of unfractionated preparations were also calculated by them, but, owing to the scattered values obtained, no definite conclusion are to be expected. Other recorded values of molecular weight calculated from sedimentation constant (or sedimentation coefficient) by assuming that the molecule is spherical would not be worth mentioning, as it was found by the present study as well as by Butler et al. that the frictional ratio of histones always showed a high value of about 2. Of interest is the view of Luck et al.[20] that the monomeric molecular weight of any fraction of histones is as low as about 10,000. They came to this conclusion by measurements of osmotic pressure and sedimentation in the presence of guanidinium chloride or urea and by end-group analysis. However, the results of our N-terminal analysis[22] have failed to support their view, although more work is necessary to see if the dissociation of our preparation does occur.

As the preparation used in this study had been obtained under milder conditions, and measurements were made under a suitable condition using ultracentrifugally homogeneous samples, it is more likely that the values of molecular weight obtained in this investigation would be reliable. It might be noted that, although rather high values of frictional ratio were obtained with both fractions, it does not necessarily mean that they are asymmetric molecules. The results in favor of this view have been obtained as described in the previous section, and further research is now in progress.

Summary

Two fractions of calf thymus histone, histone I and histone II, were examined by sedimentation and diffusion.

Histone I was ultracentrifugally homogeneous in the buffer solutions below pH

17) J. A. V. Butler, P. F. Davison, D. W. F. James and K. V. Shooter, *Biochim. Biophys. Acta*, **13**, 224 (1954).
18) P. F. Davison, D. W. F. James, K. V. Shooter and J. A. V. Butler, ibid., **15**, 415 (1954).
19) B. Bakay, J. J. Kolb and B. Toennies. *Arch. Biochem. Biophys.*, **58**, 144 (1955).
20) J. M. Luck, H. A. Cook, N. T. Eldredge, M. I. Haley, D. W. Kupke and P. S. Rasmussen, ibid., **65**, 449 (1956).
21) H. J. Cruft, C. M. Mauritzen and E. Stedman, *Nature*, **174**, 580 (1954).
22) M. Yamasaki, N. Ui, S. Ishii, H. Fujioka, K. Iwai and T. Ando, The 29th General Meeting of the Japanese Biochemical Society, Fukuoka, Nov. 1, 1956.

7, but showed the presence of an aggregate above pH 7. The sedimentation constant (at infinite dilution) of histone I was calculated to be 2.0_3 S in an acetate buffer of pH 5.0 at ionic strength of 0.2. The homogeneity of this sample was also confirmed by diffusion experiments in the same buffer, and $5.1_3\times10^{-7}$ cm^2/sec. was obtained as the diffusion constant. Combining the values of sedimentation and diffusion constants as well as the value of partial specific volume, 0.74, the molecular weight was calculated to be 37,000.

Histone II was also found to be homogeneous by sedimentation and diffusion measurements in the acetate buffer of pH 5.0. Its sedimentation constant, diffusion constant, and molecular weight were calculated as 0.6_6 S, $7.3_0\times10^{-7}$ cm^2/sec., and 8,400, respectively.

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*On the Aggregation of Calf Thymus Histone**

By Nobuo Ui

(Received April 30, 1957)

In recent years, increasing attention has been directed toward protein interactions[1]. Indeed, it has been recognized that many reactions of biological importance, such as enzyme reactions, antigen-antibody reactions, etc., involve interaction of proteins with other proteins or other molecules. By studying the nature of these interactions, not only is it possible to interpret biological phenomena on a molecular basis, but it is also possible to obtain an information about protein structure in an aqueous environment.

It has been found that some proteins associate (aggregate) or dissociate with suitable change in their environment[2]. As these interactions which occur between protein molecules of the same kind are the most simple cases of protein-protein interactions, it would be of value to examine their nature from the physico-chemical point of view.

In the course of studies on purification and characterization of calf thymus histone, it was found that its main fraction, histone I, had a great tendency to aggregate[3]. The protein, when purified cautiously, showed a single homogeneous boundary in an ultracentrifuge at pH values below 7[4,5], but a heavier component appeared when the solution was kept at room temperature, and its amount varied markedly with change in the conditions of pretreatment and measurements. The present author was interested in this finding and studied the aggregation reaction systematically, the results of which are reported in this paper.

Experimental

Material.—The sample used in this study was histone I, i.e., the main fraction of calf thymus histone. The method used for its preparation has already been described[4].

Methods.—Sedimentation and diffusion studies were carried out in the same apparatus described in the preceding paper[5]. Sedimentation measurements were made at room temperature (15—30°C), while those of diffusion were made at 20.0°C. The observed sedimentation and diffusion coefficients were always corrected for the values in water at 20.0°C.

* A part of this work was read before the Symposium on Protein Structure held in Tokyo on November 5, 1955.

1) I. M. Klotz, in H. Neurath and K. Bailey, "The Proteins," New York, Vol. I, p. 727 (1953).

2) D. F. Waugh, *Advances in Protein Chem.*, **9**, 325 (1954).

3) N. Ui, *Biochim. Biophys. Acta.*, **22**, 205 (1956).

4) N. Ui, ibid. in press.

5) N. Ui, This Bulletin, **30**, 801 (1957).

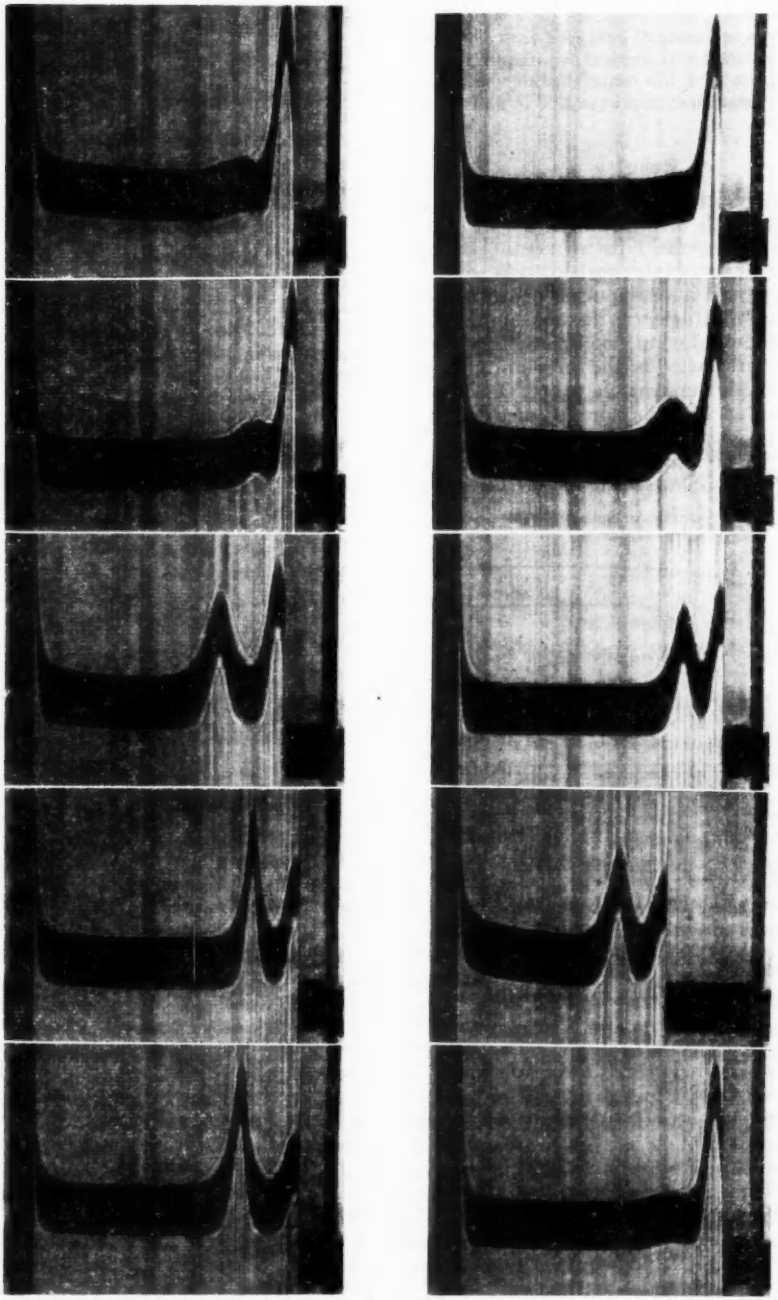

Fig. 1. Sedimentation patterns of aged solutions of histone I at pH 5.0 and ionic strength of 0.1
Left : Solutions were kept at 30°C in the presence of sodium sulfate (2/300 M) for periods of 30 min., 3, 24, 48 and 168 hr. from top to bottom.
Right : Upper 4 patterns were obtained after aging at 30°C in the absence of sulfate ions for 30 min., 28, 76 and 168 hr. Extreme bottom pattern was obtained after 7 days' keeping at 0°C in the presence of sulfate ions.
Sedimentation proceeds from right to left.

Electrophoretic measurements were made in a Tiselius-type apparatus[6] equipped with a Philpot-Svensson's schlieren optical system[7], and a medium cell with a tall center section[8] was used.

Viscosity was measured at 30.0°C in an Ostwald viscosimeter.

Results

A. Aggregation at 30°C at pH 5.0 and Ionic Strength 0.1.—When a 1 per cent. solution of histone I in an acetate buffer of pH 5.0 at ionic strength of 0.1 was allowed to stand at 30°C and examined in an ultracentrifuge, a faster component with a sedimentation coefficient of 8—10 S was observed besides the original component of this preparation (about 2 S) as is shown in Fig. 1. The amount of the faster component increased slowly with time of aging at 30°C, and reached 55 per cent. of the total protein (see Fig. 2, curve II).

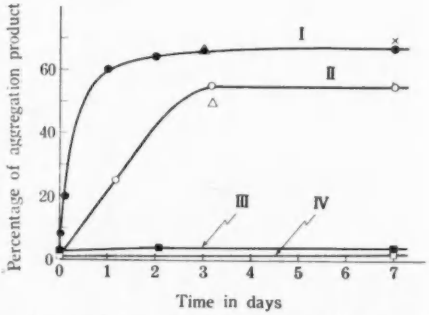

Fig. 2. Increase of the aggregation product with time at pH 5.0 and ionic strength of 0.1.
Curve I: 30°C, in the presence of sulfate ions.
Curve II: 30°C, in the absence of sulfate ions.
Curve III: 0°C, in the presence of sulfate ions.
Curve IV: 0°C, in the absence of sulfate ions.

Sulfate ion was found to be an effective aggregating agent; if an acetate buffer of the same pH and total ionic strength as before but containing sodium sulfate (2/300 M) was used as a solvent, the faster component appeared more rapidly and the amount reached 67 per cent. after one or two days (see Fig. 2, curve I). Some of the sedimentation patterns are shown in Fig. 1.

In contrast with the observation at 30°C, the aggregation did not proceed appreciably at 0°C (see curves III and IV in Fig. 2). Although a small amount (2—3 per cent.) of aggregation was observed in this experiment, it would be due to the high temperature (nearly 30°C) at which the sedimentation was performed. In fact, no faster boundary was found when another solution of histone I was kept for 25 days at 0°C and examined at a lower temperature.

In Fig. 3 is shown the sedimentation coefficient of the faster component as a function of its amount. It was found that the more the aggregation proceeded, the higher was the sedimentation coefficient obtained.

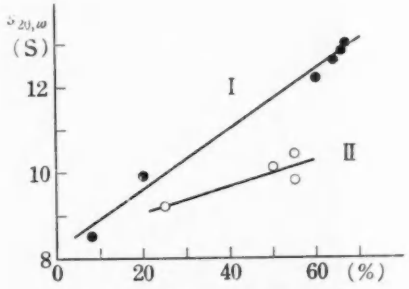

Fig. 3. Relation of sedimentation coefficient of the aggregate with its amount.
Curve I: Aged at 30°C in acetate-Na_2SO_4 buffer of pH 5.0 at ionic strength of 0.1.
Curve II: Aged at 30°C in acetate buffer (sulfate-ion free) of pH 5.0 at ionic strength of 0.1.

The aggregation was not reversed by lowering the temperature. Therefore, when solutions of aggregated histone were examined before and after keeping them at 0°C for 4 days, the amount of aggregate was found to be nearly the same (see marks △ in Fig. 2)*. It was also found that the aggregate, which had been produced in the solution containing sodium sulfate, did not dissociate when sulfate ions were removed by addition of barium chloride (see mark × in Fig. 2).

B. Effect of Variation in Temperature.—Fig. 4 shows the amounts of aggregate formed when a 1 per cent. solution

6) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).
7) J. St. L. Philpot, *Nature*, **141**, 283 (1938); H. Svensson, *Kolloid-Z.*, **87**, 181 (1939).
8) L. G. Longsworth, *Chem. Revs.*, **30**, 323 (1942).

* In Fig. 2, the amounts of aggregate are plotted against the time kept at 30°C. As the aggregation was not reversed by cooling, it was not necessary to perform sedimentation experiments at the same temperature as in the case of the aging. A lower temperature was rather desirable to prevent progress of aggregation during the measurements.

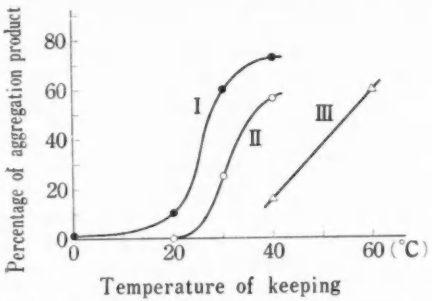

Fig. 4. Effect of temperature upon aggregation.
Curves I and II: Aged for 24 hrs. at pH 5.0 and ionic strength of 0.1, in the presence and absence of sulfate ions.
Curve III: Aged for an hour at pH 5.0 and ionic strength of 0.1, in the absence of sulfate ions.

of histone I in acetate buffers (pH=5.0, ionic strength=0.1) with and without sodium sulfate (2/300 M) were kept at various temperatures. Curves I and II were obtained by keeping the solutions for 24 hours, while curve III was obtained after one hour's keeping.

It was clear that the higher the temperature, the higher the extent of aggregation. At 20°C, histone I did not aggregate during 24 hour's keeping in the absence of sulfate ion, but the aggregation occurred by aging for a longer time (8 per cent. after 4 days' keeping). A lower temperature than 20°C was necessary to keep the solution free from the aggregate for long time.

At higher temperatures, it was found that, not only was the extent of aggregation increased, but also the reaction rate was accelerated. For instance, the initial slope of the amount of aggregate vs. aging time curve was steeper at 40°C than at 30°C. At 40°C, the amounts of aggregate reached 82 per cent. in the presence of sulfate ions and 62 per cent. in its absence, which were higher than those obtained at 30°C.

C. Effect of Variation in Protein Concentration.—A series of histone I solutions in the same solvents as before at various protein concentrations were examined after keeping at 30°C for 24 hours.

As shown in Fig. 5, a higher concentration favored the aggregation. In the presence of sulfate ions, the extent of aggregation in 2 per cent. solution was lower than that in 1 per cent. solution, but it might be due to the decrease in

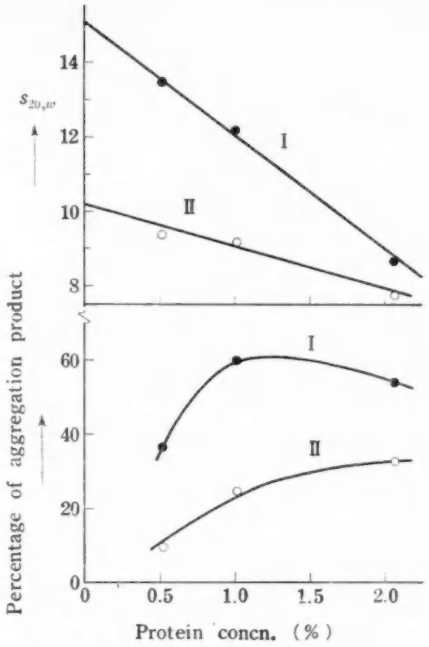

Fig. 5. Effect of protein concentration upon aggregation.
Aged at 30°C for 24 hrs. at pH 5.0 and ionic strength of 0.1, in the presence (curve I) or absence (curve II) of sulfate ions.

the molar ratio of sulfate to the protein with increase in protein cencentration, since the concentration of sodium sulfate was always kept constant (2/300 M) in this experiment.

D. Effect of Variation in Ionic Strength of the Solution.—Effect of ionic strength was studied at pH 5.0 with 1 per cent. solutions of histone I. The concentration of sodium sulfate, if present, was kept always constant at 2/300 M.

The amounts of aggregate produced during 24 hours' aging at 30°C are plotted against ionic strength of the solution in Fig. 6. It was shown that high ionic strength favored aggregation. In the absence of sulfate ions aggregation hardly occurred below ionic strength of 0.05. It was also indicated that the effect of sulfate ion on aggregation was accentuated at lower ionic strengths.

The temperature dependency of this aggregation was again noted; at 0°C the aggregate was not produced appreciably even if the ionic strength was higher and sulfate ions were present (see Fig. 6, curve III).

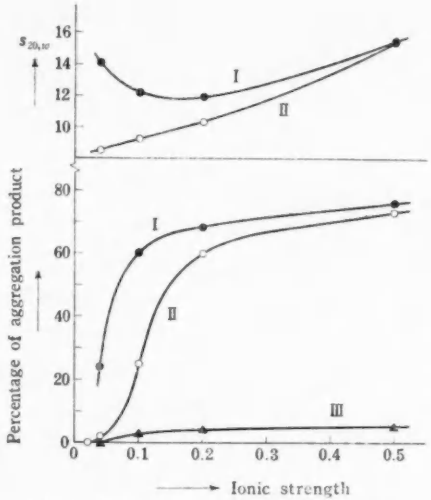

Fig. 6. Effect of ionic strength upon aggregation.
Aged at 30 C for 24 hr. at pH 5.0 in the presence (curve I) or absence (curve II) of sulfate ions. Curve III is that of aged solutions at 0 C.

The sedimentation coefficient of the aggregate also depended upon the ionic strength of the solution (see upper figure in Fig. 6), and a higher value was obtained at a higher ionic strength. In the case of solutions containing sulfate ions, however, sedimentation coefficient vs. ionic strength curve showed a minimum, suggesting the complicated effect of sulfate ion upon aggregation.

E. Effect of Adding Sodium Sulfate.—In view of the finding that the aggregation of histone was greatly accelerated by the presence of sulfate ions, a different amount of sodium sulfate was added to the solution of histone I (pH 5.0) and the effects were compared.

When sodium sulfate was added to a concentration higher than 2/300 M, no appreciable change was observed in the amount of the aggregate formed after 48 hours' aging at 30°C, but the sedimentation coefficient of the aggregate was definitely higher at higher concentration of sulfate ions; for instance, 17.0 S was obtained for 5/300 M sodium sulfate instead of 11.8 S for 2/300 M (total ionic strength was equal to 0.1).

Even a slight addition of sulfate ions was effective for the aggregation. Therefore, histone I sulfate aggregates more easily than its chloride. However, it might be noted that the aggregation of histone I chloride in the acetate buffer (without sodium sulfate) was not due to a slight contamination of sulfate ions in the solution; a curve similar to curve II in Fig. 2 was obtained when the acetate buffer containing barium chloride was used and aged at 30°C.

F. Effect of Variation in pH.—Dependence of the aggregation of histone I upon pH was studied by using buffer solutions of constant ionic strength of 0.1*. Protein concentration was always kept at 1 per cent. and the concentration of sodium sulfate, if present, was 2/300 M.

The amount of the aggregate formed after

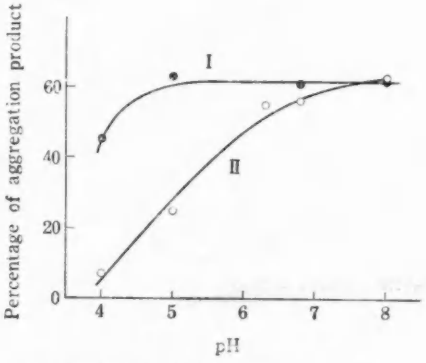

Fig. 7. Effect of pH upon aggregation.
Aged at 30°C for 24 hr. at ionic strength of 0.1 in the presence (curve I) or absence (curve II) of sulfate ions.

24 hours' aging at 30°C is plotted in Fig. 7 as a function of pH. It was noted that higher pH favored aggregation in absence of sulfate ions, but the amount of aggregate found was almost independent of pH values when sodium sulfate was added.

It was also found that some electrolytes caused marked influence upon the aggregation; a gel-like precipitate formed after keeping histone I at 30°C in a phosphate buffer of pH 7.7 or in a veronal buffer of pH 9.0.

As described in the preceding paper[6], histone I showed the presence of a faster component when sedimentation experiment was performed in an alkaline solution above pH 7 except in tris(hydroxymethyl)aminomethane buffer, even if the solutions had not been exposed to a higher temperature. In some cases, especially when pH was 9—10, a precipitate was

* The Following buffers were used in this study: pH 4.0 and pH 5.0, acetate buffer; pH 6.3, NaCl solution (unbuffered); pH 6.8, cacodylate buffer; pH 8.0, tris-(hydroxymethyl)aminomethane buffer.

formed by several days' keeping at 0°C. It was also found that the aggregate disappeared when the solution was soon brought to pH 5.0, so the reversibility of aggregation was suggested.

G. Reversibility of the Aggregation Reaction.—As stated before (see section A), the aggrgation was not reversed by lowering the temperature. However, a disaggregation occurred when an aggregated solution was brought to a lower ionic strength by dialysis. Results are shown in Table I.

TABLE I

DISAGGREGATION OF AGGREGATE BY DIALYSIS

pH : 5.0 (acetate buffer) ;

Protein concn. : ca. 1%

Aging			Amount of aggregate after dialysis against buffer with ionic strength of		
Ionic strength	Time at 30°C	Aggregate formed	0.02	0.04	0.1
1.0	72 hr.	73%	—	—	30%
0.1	72	50	—	24%	—
0.1	96	55	15%	—	(15%)*
0.1	24	20	—	—	20%**

* This value was obtained on the solution which had been re-adjusted to ionic strength of 0.1 after being brought to ionic strength of 0.02 by dialysis.

** The solution was once brought to pH 2 by addition of hydrochloric acid and then dialyzed against the acetate buffer of ionic strength 0.1.

H. Molecular-kinetic Properties of the Aggregates.—In order to obtain some information on the size and the shape of the aggregates, sedimentation and diffusion experiments were made with 1 per cent. solution of histone I in an acetate buffer of pH 5.0 at ionic strength of 0.2. The solution had been kept for 4 days at 30°C in the presence and the absence of sodium sulfate (2/300 M). The amount of aggregate formed did not decrease with dilution.

In Fig. 8 is plotted the sedimentation coefficient of the aggregate against concentration, and the sedimentation constant at infinite dilution was calculated by extrapolation.

Diffusion curves markedly deviated from Gaussian curves, as the solution also contained the unaggregated protein. However, since the diffusion coefficient of the unaggregated histone I is known[5], the diffusion coefficients of the aggregates could be calculated, although not accurately, making use of the weight-averaged diffu-

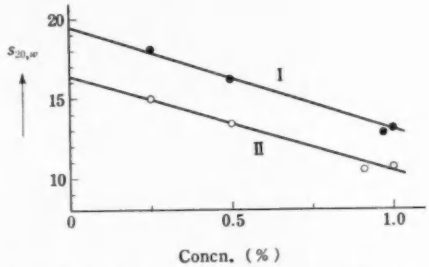

Fig. 8. Concentration dependency of sedimentation coefficient of aggregate at pH 5.0 and ionic strength of 0.2.
Curve I : The aggregate was prepared by aging at 30°C for 4 days in the presence of sodium sulfate (2/300 M).
Curve II : The aggregate was prepared in the same way but in a sulfate ion-free buffer.

sion coefficients (obtained by the second-moment method) as well as the composition of the solutions determined by sedimentation analysis. The results are shown in Table II. As the diffusion patterns did not show concentration dependency of the

TABLE II

DIFFUSION DATA ON AGED SOLUTION OF HISTONE I

Buffer	Aggregate formed (%)	Weight-averaged diffusion coefficient ($D_{20,w} \times 10^7$ cm²/sec.)	Diffusion coefficient of aggregate ($D_{20,w} \times 10^7$ cm²/sec.)
Acetate	66	1.3_3	1.4_7
Acetate-Na_2SO_4	83	1.6_1	1.7_5

diffusion coefficients of the constituents, the values were taken as diffusion constants.

The molecular weight of the aggregate formed either in the presence or the absence of sulfate ions was thus calculated by combining the values of sedimentation and diffusion constants and by assuming that the partial specific volume of the aggregate was the same as unaggregated histone I. In both cases, a value of 1.1×10^6 was obtained (see Table III) and it was found that the aggregate formed in these solutions consisted of about 30 molecules of histone I.

The frictional ratio of the aggregate nearly agreed with that of unaggregated histone. A similar result was obtained when the change in viscosity with time was measured at 30°C. In the absence of sulfate ions (pH=5.0, ionic strength=0.1), η_r

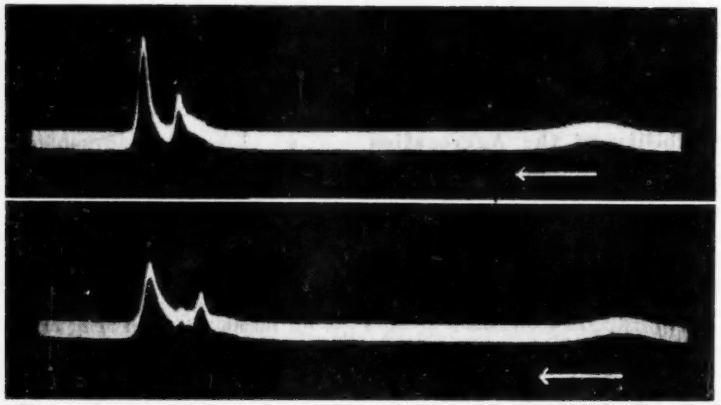

Fig. 9. Electrophoretic patterns of unaggregated (A) and aggregated (B) solutions of histone I in the acetate buffer of pH 5.0 at ionic strength of 0.1. Only ascending patterns are shown.

TABLE III
MOLECULAR-KINETIC DATA ON AGGREGATED HISTONE I

Buffer	$s_{20,w}$ (S)	$D_{20,w} \times 10^7$ (cm²/sec.)	M	f/f_0
Acetate	16.4	1.3_3	1,150,000	2.30
Acetate-Na_2SO_4	19.7	1.6_1	1,140,000	1.91

(viscosity ratio) of 1.18 per cent. solution increased slightly, i.e., from 1.20 to 1.35 after 120 hours. Similarly, when sulfate ions were present, η_r of 1.16 per cent. solution changed from 1.18 to 1.25 after 32 hours' keeping.

I. Electrophoretic Behavior of the Aggregate.—When the aggregation of histone I took place, some change in electrophoretic patterns were observed. An example is shown in Fig. 9.

Although even unaggregated histone I did not show a single boundary (see Fig. 9 A), the electrophoretic patterns became more complex when aggregation occurred (Fig. 9 B); the patterns shown in Fig. 9 were obtained in the acetate buffer of pH 5.0 at ionic strength of 0.1 using the solution which had not been exposed to a high temperature and the solution which had been kept at 30°C for 2 days (the latter solution contained the aggregate about 24 per cent.). It was found that the fastest boundary in the case of the aggregated solution moved to the cathode faster than the fastest component of unaggregated histone I*. A similar variation in electrophoretic pattern with aggregation was observed at other ionic strengths, at a different temperature (25°C), or in the presence of sulfate ions. Therefore, it was concluded that the aggregate had an electrophoretic mobility higher than unaggregated histone at pH 5.0.

* The electrophoretic mobility (descending) of the fastest component was calculated as 8.0×10^{-5} cm²/sec. volt in the absence of the aggregate, while 8.6×10^{-5} in the case of the aggregated solution.

Discussion

It is indicated by the results of ultracentrifugal studies described above that histone I produces a rapidly sedimenting component with aging at a temperature higher than about 20°C at the expense of the original component of this protein (2 S), provided that the ionic strength of the solution is not too low. As the molecular-kinetic studies had shown that the size of the faster component was definitely larger than the original protein, the reaction occurring would be due to an aggregation.

The sedimentation coefficient of the newly formed component increased continuously as the reaction proceeded and varied with aging conditions. In view of this finding, the term "aggregation" was used throughout this paper in preference to "association" (for the definition of "aggregation" and "association", see the reference[2]). However, the fairly regular nature of the reaction was suggested, as the aggregate always showed a single boundary with moderate sharpness in an ultracentrifuge; its sedimentation coefficient and the amount were constant so far as the aging conditions were the same.

The aggregation proceeded at a rate sufficiently small to permit the estimation

of the degree of aggregation at desired levels by sedimentation analysis, and this slowness was one of the main features of this reaction. High temperature or high ionic strength was highly effective in promoting the aggregation and an equilibrium was thereby rapidly established. However, the aggregation could not be reversed by the lowering of the temperature. Disaggregation only occurred when the electrolyte concentration of the solution was reduced by dialysis*. Therefore, it seems that the process involves an equilibrium dependent on ionic strength between the monomer and the aggregate. As the rate of aggregation was slow, it might also be suggested that some modification of the protein precedes aggregation. The experimental results presented here would be accounted for if this conversion is favored by high temperature, and no significant change in the size and the shape of the molecule is assumed to occur by this process. However, as a temperature-dependent modification of calf thymus histone has not been proved, further study is needed to check this conclusion.

Of interest are the forces involved in forming the aggregate. Histone I has an isoelectric point definitely higher than pH 10.3 as revealed by electrophoresis[9]. When amino acid composition of this preparation is assumed to be the same as that of "arginine-rich histone" of Daly and Mirsky[10], it follows that histone I has about 78 positively charged and 21 negatively charged groups per molecule at pH 5.0 (net charge=+57). Therefore, strong repulsion between molecules is expected to exist at this pH, and it must be overcome by specific attractive forces in oder that an appropriate interaction may occur. The contribution of van der Waals' forces or hydrogen bonds to the formation of aggregate might be considerable, although electrostatic attraction between oppositely charged groups, which are disposed appropriately, would also be responsible. In this connection, it might be pertinent to describe our finding that the aggregation was prevented by the presence of urea, which is a well-known hydrogen bond breaking agent; when a solution of histone I was kept at 30°C for 4 days in the presence of 5 M urea (pH=5.0, ionic strength 0.1), no faster sedimenting boundary was observed and only a single sedimentation boundary with the normal sedimentation coefficient of about 2 S was found after the aged solution was dialyzed against the acetate buffer of pH 5.0 and ionic strength 0.1.

It is also to be noted that the suitable configuration of the protein molecule would be required in order that the aggregate might be stabilized by forming a number of bondings due to short range attractive forces (such as hydrogen bonds or van der Waals' forces). Since an electrostatic, repulsive force between charged groups is less prominent at a high ionic strength or a high pH, the aggregated form must become favorable when ionic strength or pH is elevated.

The specific effect of sulfate ions on promoting the aggregation should be emphasized. As the electrophoretic mobility of histone I (unaggregated) at pH 5.0 was found to be markedly diminished by the addition of sodium sulfate[9], it is clear that a large number of sulfate ions are bound with one molecule of histone I. Accordingly, the electrostatic repulsive force between molecules would not be strong enough when sulfate ions are present. It might be also probable that sulfate ions in some manner stabilize the modified form of histone I, which was assumed in the above discussion to be an intermediate in the aggregation reaction. Another bivalent anion, dibasic phosphate ion, was also found to be an effective aggregating agent.

The aggregation product was found to consist of fairly a large number of histone I molecules; i.e., the number of units was about 30 when calculated for the preparation aged at 30°C for 4 days in the presence or the absence of sulfate ions (pH=5.0 and total ionic strength=0.2). Although exact shape of the aggregate is not known, the frictional ratio, which depends upon both shape and hydration, was found to be similar to that of the unaggregated histone I. Electrophoretic mobillty at pH 5.0 only slightly increased with the aggregation. Thus, no significant change except size was found to occur with aggregation. However, it was noticed that histone I solution solubilized sparingly water-soluble materials, such as dimethylaminoazobenzene, especially under conditions in which the aggregation is favorable (unpublished

* The aggregate formed at pH's higher than at low temperature disaggregated when the solution was brought to lower pH's. However, the aggregation in an alkaline solution became more or less irreversible after keeping the solution for a longer time.

9) N. Ui, This Bulletin, in press.

10) M. M. Daly and A. E. Mirsky, *J. Gen. Physiol.*, **38**, 405 (1955).

data). A similar phenomenon has often been reported in an aqueous solution of soap or detergent[11], and is thought to be due to the formation of micelles through the mutual association of hydrophobic and hydrophilic groups in which a sparingly water-soluble material is taken in. On the analogy of this interpretation, it might be possible that the molecules of histone I are specifically arranged in the structure of the aggregate to take in these materials. However, as the interpretation is a matter of speculation, more work is necessary as to the structure of the aggregate.

Since the aggregation of histone I proceeds even at room temperature, especially when the ionic strength of the solution is high, the temperature should be kept as low as possible during the preparation of histone. At pH's higher than 7, the aggregation occurs even if the solution is kept cold and becomes more or less irreversible with time. Therefore, the method for the preparation of histone which takes advantage of its precipitability in an alkaline solution is not recommended. A faster sedimenting component of histone with a sedimentation coefficient much higher than 2 S, which has often been reported by other investigators (see D scussions in the preceding paper[9]) seems to be due to the aggregation that occurred during the isolation of the protein or in the solution in which measurement was performed.

In agreement with the present study, the aggregation of histone has also been pointed out by Cruft, Mauritzen and Stedman[12] and by Davison, James, Shooter and Butler[13]. The former workers came to this conclusion by diffusion measurements which were performed at various pH's. Davison et al. took advantages of sedimentation and other methods, but no detailed data have been reported. They also interpreted the complicated electrophoretic patterns, which were obtained with unfractionated histone at various pH values, by assuming a complex formation between different fractions of histone, and suggested that the greater part of the aggregation reaction involves both 2 S and 1 S component. However, as the electrophoretic patterns were highly complicated[9], more extensive work seems necessary to confirm Davison et al's view.

It is of interest to compare the results of the present study with those of similar studies on other proteins. While association or aggregation occurs with several proteins, it has been known that, only in a few cases of these interactions, an equilibrium is attained at such a slow rate as described in this paper. Johnson et al.[14] have shown that an association-dissociation equilibrium exists in the solution of arachin, a seed globulin in peanut, and that the rate of association or dissociation was slow. It was also shown that pH and ionic strength as well as sulfate ion strongly changed the state of equilibrium and reaction rate[15]. As to the aggregation of conalbumin with acid, Cann et al.[16] showed that the ability of positively charged conalbumin molecules to aggregate resulted from structural modification when the protein was exposed to acidic media. The remarkable effect of sulfate ion upon this aggregation was also stressed. Specific effects of some electrolytes upon a more rapidly attained association-dissociation equilibrium have been reported with hemocyanin[17], insulin[18] and salmine[19].

Summary

The aggregation reaction of histone I, i. e., the main fraction of calf thymus histone, was studied by means of sedimentation, diffusion, viscosity measurement and electrophoresis.

The aggregation was found to proceed usually at a temperature higher than 20°C, although it occurred at pH's above 7 even at 0°C. High temperature, pH, ionic strength or protein concentration promoted the reaction. It was also found that sulfate ion was an effective aggregating agent.

The aggregation was not reversed by lowering the temperature, but disaggregation occurred when the ionic strength of the solution containing the aggregate was reduced by dialysis.

The aggregate was rather large in size.

11) H. B. Klevens, *Chem. Revs.*, **47**, 1 (1950).
12) H. J. Cruft, C. M. Mauritzen and E. Stedman, *Nature*, **174**, 580 (1954).
13) P. F. Davison, D. W. F. James, K. V. Shooter and J. A. V. Butler, *Biochim. Biophys. Acta*, **15**, 415 (1954).
14) P. Johnson and E. M. Shooter, ibid., **5**, 361 (1950); P. Johnson, E. M. Shooter and E. K. Rideal, ibid., **5**, 376 (1950).
15) P. Johnson and F. J. Joubert, *J. Polymer Sci.*, **7**, 605 (1951).
16) J. R. Cann and R. A. Phelps, *Arch. Biochem. Biophys.*, **52**, 48 (1954); R. A. Phelps and J. R. Cann, ibid., **61**, 51 (1956).
17) S. Brohult, *J. Phys. & Colloid Chem.*, **51**, 206 (1947).
18) E. Fredericq and H. Neurath, *J. Am. Chem. Soc.*, **72**, 2684 (1950).
19) N. Ui, *Repts. Institute Science and Technol., Univ. of Tokyo*, **8**, 255 (1954).

It consisted of about 30 molecules of histone I under certain aging conditions (pH 5.0) and had nearly the same frictional ratio as that of the monomer. Electrophoretic mobility slightly increased with aggregation at pH 5.0.

On the basis of the data presented, a possible mechanism in forming the aggregate was discussed.

The author wishes to express his sincere thanks Professor Itaru Watanabe for his valuable suggestions and encouragement. His thanks are also due to the Research Laboratory of Precision Machinery and Electronics, Tokyo Institute of Technology, for the kind permission to use their Spinco ultracentrifuge. The expense of this research was partly defrayed by a Grant in Aid for Scientific Research from the Ministry of Education, to which the author's thanks are due.

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Electrophoretic Studies of Calf Thymus Histone

By Nobuo Ui

(Received May 7, 1957)

As was reported in the previous papers[1,2], calf thymus histone consists of two fractions (histones I and II). It was also shown that both were ultracentrifugally homogeneous. However, electrophoretic investigation on these fractions revealed that neither of them was homogeneous.

The present paper deals with the electrophoretic properties of histone I, the main component. A few experiments with histone II are also reported and the results are compared with those of histone I. Although electrophoretic heterogeneity of calf thymus histone has also been proved by other workers[3-7] the present experiments showed the presence of more components than had ever been reported before.

Experimental

Materials.—Two fractions of calf thymus histone used in this investigation, i.e., histones I and II, were prepared by the method described in a previous paper[1]. The preparation of histone I mainly used was obtained from the extract of isolated nuclei with 0.2 N sulfuric acid after removing histone II by extraction with 0.1 N sulfuric acid.

Methods.—Electrophorsis was carried out in a Tiselius-type apparatus[8] equipped with a Philpot-Svensson's shlieren optical system[9]. Prior to electrophoresis, each preparation was dissolved in a desired buffer and dialyzed against a large quantity of the buffer in a cold room which was maintained approximately at 0°C, although, in some experiments, the dialysis was omitted or performed at 25°C.

The pH value of some buffers, such as ammonium chloride ammonia buffers, markedly varied with temperature. Accordingly, pH measurements were made at a temperature nearly the same as that of electrophoresis. Acetate buffers used in most of the experiments did not show any change in pH with temperature.

In most cases, runs were made at a constant temperature of approximately 0°C (at 0.2°C or slightly higher), but some experiments were made at 25.0°C to see the effect of temperature.

A medium electrophoretic cell with a tall center section was used throughout this investigation, and electrophoresis was continued usually until the distance of migration of the fastest boundary reached 6 cm. or more, as it was found that separation of boundaries was poor when the migration distance was shorter.

Specific electric conductivity of the solution was measured in a bath maintained at 0.0°C (or 25.0°C when electrophoresis was carried out at 25.0°C). Therefore, the electrophoretic mobility

1) N. Ui, *Biochim. Biophys. Acta*, in press.
2) N. Ui, This Bulletin, **30**, 801 (1957).
3) J. Grégoire and M. Limozin, *Bull. Soc. Chim. Biol.*, **36**, 15 (1954).
4) J. A. V. Butler, P. F. Davison, D. W. F. James and K. V. Shooter, *Biochim. Biophys. Acta*, **13**, 224 (1954).
5) P. F. Davison, D. W. F. James, K. V. Shooter and J. A. V. Butler, ibid., **15**, 415 (1954).
6) H. J. Gruft, C. M. Mauritzen and E. Stedman, *Nature*, **174**, 580 (1954).
7) J. M. Luck, H. A. Cook, N. T. Eldredge, M. I. Haley, D. W. Kupke and P. S. Rasmussen, *Arch. Biochem. Biophys.*, **65**, 449 (1956).
8) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).
9) J. St., L. Philpot, *Nature*, **141**, 283 (1938); H. Svensson, *Kolloid-Z.*, **87**, 181 (1939).

(u) obtained was thought to be the value at 0.0 C (or 25.0°C).

The greatest difficulty encountered in this investigation was due to the fact that solutions of histone solubilized some kinds of water-insoluble material. Thus, when ground surfaces of flanged plates at each end of cell segments were greased as usual with vaseline or silicone grease (Dow Corning's high vacuum silicone grease), there occurred serious disturbance in initial boundaries formed between the protein solution and buffer at the beginning of the experiments. The difficulty was overcome by using silicone oil with high viscosity (Shin-Etsu Silicone Oil KF 96, 100,000 cs.). The author is indebted to Dr. K. Hayashi, Shin-etsu Chemical Co., for his kind supply of various preparations of silicone oil.

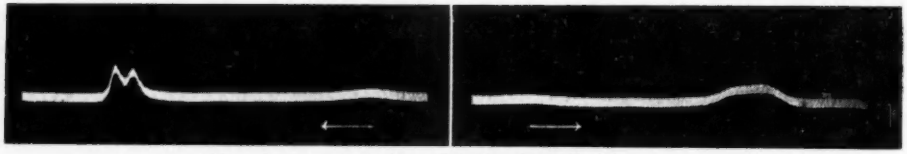
Results and Discussion

A. Electrophoretic Behavior of Histone I at pH 5.0.—The preparation of histone I was examined by electrophoresis in acetate buffers of pH 5.0 at various ionic strengths. The protein concentration was almost at one per cent. It was found that at least three components with similar electrophoretic mobilities migrated towards the cathodes, although separation was poor at higher ionic strength. Some electrophoretic patterns are shown in Fig. 1 (A—C).

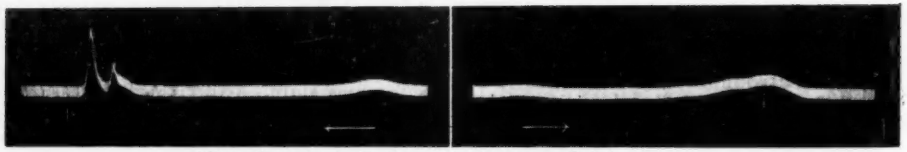
It was also noticed that the electrophoretic composition varied markedly with change in ionic strength. The apparent relative concentrations of the fastest migrating component computed from the ascending pattern was 48 per cent. at ionic strength of 0.2, while at ionic strength of 0.05 a much higher value, 73 per cent., was obtained. Slightly lower, but essentially the same relative concentration was obtained from the corresponding descending pattern, although the value was not accurate owing to the broadness and overlapping of the boundaries.

A similar change in apparent relative concentrations was also observed when the protein concentration was lowered from one to 0.5 or 0.3 per cent. at constant ionic strength of 0.1.

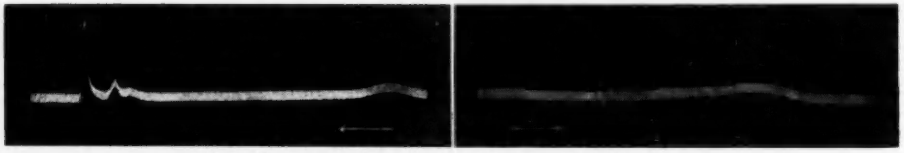
As the change in electrophoretic patterns with change in ionic strength or protein concentration was astonishingly great, it was felt, at first, that more than



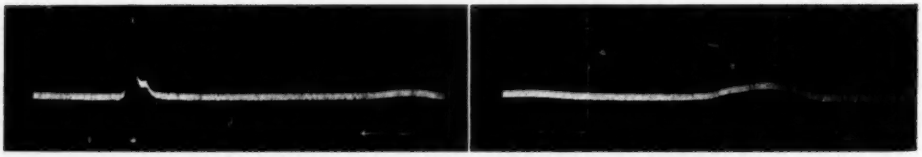
(A) : In acetate buffer of ionic strength 0.2.



(B) : In acetate buffer of ionic strength 0.1.



(C) : In acetate buffer of ionic strength 0.05.



(D) : In acetate—Na_2SO_4(5/300 M) buffer ionic strength 0.1.

Fig. 1. Electrophoretic patterns of histone I at pH 5.0 (0°C). Ascending patterns on the left and descending patterns on the right.

one form of the protein existed in solutions and the state of equilibrium was sensitive to ionic strength or protein concentration. However, this view was soon found to be incorrect. When the deviation of apparent composition from the true composition was estimated with the aid of the moving-boundary theory developed by Dole[10], it was found that the apparently abnormal phenomenon could be accounted for on the basis of the so-called boundary anomalies[11], which were greatly exaggerated in the case of histone I solution; large errors in the apparent analysis would be made in the case of a mixture with nearly the same mobilities[12] as encountered in the present experiments, since the contribution of superimposed gradients of buffer ions and foreign proteins becomes great in such a system.

With the aid of the Dole's equation[10], change in the theoretical, apparent composition with change in ratio of protein concentration to ionic strength (c/μ)* was calculated for hypothetical mixtures of two components with the relative mobilities (r) of 0.28 and 0.26 which corresponded to the estimated mobility values of the two main components (faster components) of histone I. The net positive charge on each component was assumed to be 27 or 25 per molecule with a molecular weight of 37,000**. In addition, it was assumed, for the sake of simplicity, that the solution contained only sodium ($r=1.00$) and acetate ($r=-0.79$) ions besides the proteins. In so far as it is permissible to neglect the minor, third component in histone I observed in the case of lower ionic strength, such a solution would be a simplified system representing a histone I solution at pH 5.0. The refractive-index increment of each constituent in the solution was appropriately assumed.

The results of calculations for mixtures containing 30 and 40 per cent. of the fastest component are shown in Fig. 2. It is clear that the dependency of the apparent concentration upon the ratio of protein concentration to ionic strength is very great. In spite of many assumptions

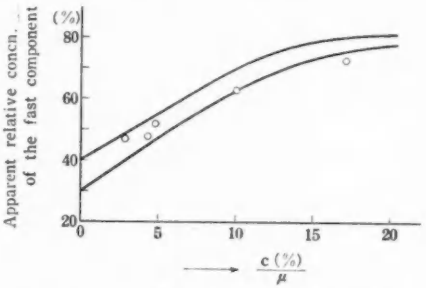


Fig. 2. Variation of apparent relative concentration of the fastest component of histone I with the ratio of protein concentration to ionic strength.

involved in theory and calculation, the theoretical curve for the solution containing 30 or 40 per cent. of the faster component fitted fairly well with the experimental points, which are represented as circles in this figure. Thus, the heterogeneity of histone I was confirmed and the true percentage of the fastest component was estimated to be 30—40 per cent. of the total proteins.

As might be expected from the results described above, it was shown that exposure of the solution to high ionic strength had no effect; when a 2 per cent. solution in the acetate buffer of ionic strength 0.2 (pH 5.0) was kept at 0°C for one day and subjected to electrophoresis without dialysis after being diluted with the same volume of distilled water, the electrophoretic pattern was found to be nearly the same as that obtained at ionic strength of 0.1.

A small variation in the electrophoretic composition of components was found with different preparations of histone I. It was thought that different procedures for extraction had caused this variation.

In Table I are shown the electrophoretic mobility data of histone I at pH 5.0. It should be noticed that the values, except those of the fastest component calculated from descending patterns, are only apparent ones, since the specific conductivity of

10) V. P. Dole, *J. Am. Chem. Soc.*, **67**, 1119 (1945).
11) L. G. Longsworth, *J. Phys. & Colloid Chem.*, **51**, 171 (1947).
12) C. Hoch-Ligeti and H. Hoch, *Biochem. J.*, **43**, 556 (1948).

* An apparent composition will agree with a true composition when the ratio of protein concentration to ionic strength becomes equal to zero.

** The net charge on either of two main components of histone I was calculated from its electrophoretic mobility by using Gorin's equation[13] for a long cylinder. Taking the results of sedimentation and diffusion studies[2] into consideration, a cylinder of 7.1 Å in diameter and 290 Å in length was assumed for both of the components. However, as it seems unlikely that the molecules are so elongated (see discussion in a previous paper[2]), the values of charges thus obtained would be only tentative. Calculated from amino acid composition using the data of Daly and Mirsky[14] on their arginine-rich histone, the valency was 57 at pH 5.0 and was almost twice as high as that adopted in the present study.

13) H. A. Abramson, L. S. Moyer and M. H. Gorin, "Electrophoresis of Proteins," New York (1942).
14) M. M. Daly and A. E. Mirsky, *J. Gen. Physiol.*, **38**, 405 (1955).

TABLE I
ELECTROPHORETIC MOBILITY DATA ON HISTONE I AT 0°C IN ACETATE BUFFERS OF pH 5.0

Ionic strength	Concn. (%)		$u \times 10^5$ cm²/sec. volt Comp. 1	Comp. 2	Comp. 3
0.2	0.90	Des.	6.4	5.8	—
		As.	7.0	6.6	—
0.1	1.00	Des.	8.0	6.9	—
		As.	9.1	8.4	5.6
0.1	0.51	Des.	8.3	7.5	6.0
		As.	9.0	8.1	7.7
0.1	0.29	Des.	8.5	7.7	6.1
		As.	8.9	7.9	7.3
0.05	0.85	Des.	9.4	7.6	5.9
		As.	11.5	10.4	9.9

protein solutions was used for these calculations.

B. Effect of Sulfate Ion on the Electrophoretic Behavior of Histone I.—It has been found[15] that the aggregation of histone I was greatly accelerated when histone I sulfate was used or when the sulfate ion was added to the solution. Therefore, the effect of the sulfate ion upon electrophoretic behavior of histone I was studied, using acetate buffers of pH 5.0 containing sodium sulfate.

As shown in Table II, it was found that electrophoretic mobility markedly decreased when the sulfate ion was present. When sodium sulfate was added to one-half of the total ionic strength of 0.1, the mobility was about two-thirds of the value

TABLE II
ELECTROPHORETIC MOBILITY DATA ON HISTONE I AT 0°C IN ACETATE BUFFERS CONTAINING SODIUM SULFATE

Ionic strength	Concn. of sodium sulfate		$u \times 10^5$ cm²/sec. volt Comp. 1	Comp. 2
0.2	1/30 M	Des.	4.3	3.9
		As.	4.9	4.6
0.1	5/300 M	Des.	5.6	4.7
		As.	6.5	6.2

in the absence of the sulfate ion. Perhaps, it would be due to the fact that a fairly large number of sulfate ions is bound to the proteins. Positively charged groups, such as guanidinium and ε-amino groups would be responsible for this interaction.

The effect of the sulfate ion on the electrophoretic composition was also observed. In accordance with the observations described in section **A**, the apparent concentration of the fastest component increased with the decrease in ionic strength, but, when compared at the same ionic strength, the value was higher than that obtained in the sulfate ion-free buffer (see Fig. 1D). The fact was again accounted for on the basis of boundary anomalies, since it was shown by the moving-boundary equation that the presence of bivalent sulfate ions accentuates the anomaly.

C. Effect of Aggregation Reaction on the Electrophoretic Behavior of Histone I.—As was shown in a previous paper[15], a component with a slightly higher mobility appeared when a solution of histone I formed an aggregate (see Fig. 9 in a previous paper[15]). Although the amount of the newly formed component could not be estimated owing to its poor separation from the fastest component in the original solution, this component was thought to correspond to the aggregation product as seen in sedimentation patterns. Although Davison, James, Shooter and Butler[5] reported that calf thymus histone contained two electrophoretically distinguishable components and that the third component appeared when the aggregation took place, it seemed that none of the boundaries described in **A** or **B** (see Fig. 1) was due to aggregation.

D. Electrophoretic Behavior of Histone I at 25°C.—To see the effect of temperature on the electrophoretic behavior of histone I, a series of experiments were carried out at 25°C using solutions of histone I which had been dialyzed at 0°C. The solvents used were acetate buffers of pH 5.0 at ionic strength of 0.2, 0.1 and 0.05, and acetate-sodium sulfate buffer of the same pH and ionic strength of 0.1.

Since the solutions used in these experiments had not been exposed to a high temperature before the initiation of electrophoresis except that they were kept at 25°C for a short period in the cell to establish a temperature equilibrium, no appreciable aggregation occurred. Only a small amount of aggregate was found in the case of solution at ionic strength of 0.2 (4 per cent.) or containing sodium sulfate (5 per cent.).

The electrophoretic patterns obtained are shown in Fig. 3. When they are compared with those obtained at 0°C (see Fig. 1), it is clear that they differ definitely at a higher ionic strength or in the presence of sulfate ions, while the difference was not large at a lower ionic strength. As these temperature dependencies could not entirely be interpreted by the spreading of boundaries due to diffusion, it was thought more likely that some changes in

15) N. Ui, This Bulletin, 30, 806 (1957).

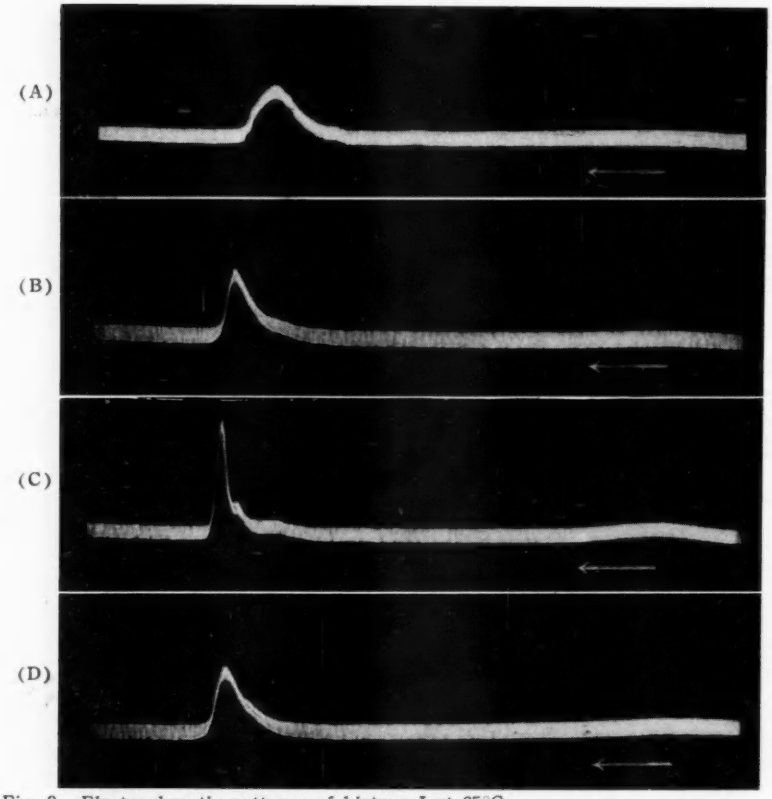


Fig. 3. Electrophoretic patterns of histone I at 25°C.
(A)—(C) : In acetate buffers of pH 5.0 at ionic strengths of 0.2, 0.1 and 0.05.
(D) : In acetate-Na_2SO_4(2/300 M) buffer of pH 5.0 at ionic strength of 0.1.
Only ascending patterns are shown.

the proteins occurred with change in temperature. However, it must also be considered that the aggregation might proceed, if not considerably, during the electrophoresis. Therefore, other interpretations might also be possible.

In other experiments, solutions of histone I aged and dialyzed at 25°C were subjected to electrophoresis. With these solutions which contained aggregate, appearance of a new component with higher mobility than that of any original component of histone I was always observed. The results agreed with the observation at 0°C described in **C**.

E. Electrophoretic Behavior of Histone I at pH's other than 5.0.—Effect of pH on the electrophoretic behavior of histone I was studied at 0°C using buffers* of various pH's at a constant ionic strength of 0.1. Although the solutions used had not been exposed to a high temperature, the aggregation product was present in the cases of alkaline solutions.

By this study, it was found that at least two components were present and they always moved toward the cathode in a range of pH's from 4.0 to 10.3. Some of the patterns are shown in Fig. 4.

Electrophoretic mobilities of the two main components (the fastest and the second fastest components) of this preparation calculated from descending patterns are plotted against pH in Fig. 5. The values decreased slowly with increase in pH below pH 9 and decreased rapidly at a higher pH region. Even at pH 10.3, however, each boundary migrated toward the cathode fairly rapidly. Although the

* The following buffers were used in this study : pH 4.0 and 5.0, acetate buffer ; pH 6.5, phosphate buffer ; pH 6.8, cacodylate or cacodylate-NaCl buffer ; pH 8.7, tris (hydroxymethyl)aminomethane buffer ; pH 9.3, veronal or veronal-NaCl buffer ; pH 10.0 and 10.3, NH_4Cl-NH_3 buffer.

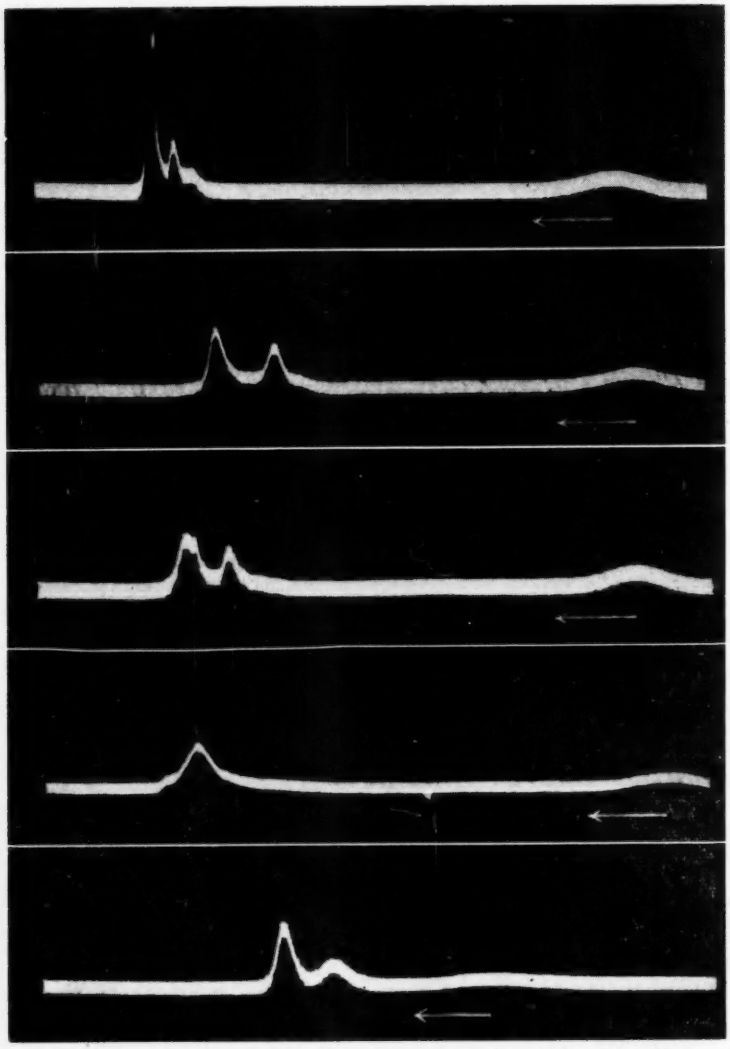


Fig. 4. Electrophoretic patterns of histone I at various pH's (0°C). The pH's are from top to bottom 4.0, 6.5, 6.8, 8.0 and 10.3, respectively. Ionic strength=0.1. Protein concn.=1%. Only ascending patterns are shown.

isoelectric point of each component could not be determined owing to the insolubility of this preparation in more alkaline solution, it was thought from the shape of the pH-mobility curve that the value was higher than pH 10.5.

At ionic strength of 0.2, similar patterns were obtained, but separation of boundaries was poorer in agreement with the results at pH 5.0.

F. Electrophoretic Behavior of Histone II.—The other fraction of calf thymus histone, histone II, was subjected to electrophoresis in acetate buffers of pH 5.0 at 0°C. As shown in Fig. 6 (A and B), one main and three minor components migrated toward the cathode. Unlike histone I, the apparent concentration of the fastest component did not vary appreciably with change in ionic strength; the values of relative concentration calculated from descending and ascending patterns were 63 and 62 per cent. at ionic strength of 0.2, and 62 and 65 per cent. at ionic strength

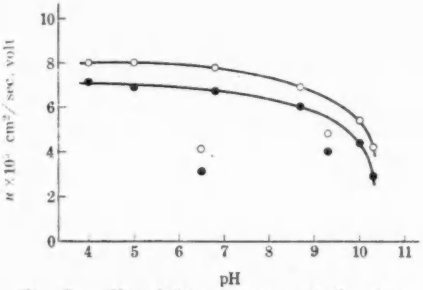


Fig. 5. pH-mobility curves of the two main components of histone I at ionic strength of 0.1 (0 C).

of 0.1. The independency of apparent concentration upon ionic strength would be due to the fact that a large mobility difference exists among the components of histone II.

The electrophoretic mobility data of histone II are shown in Table III. The mobility of the main component was even much higher than that of the fastest component in histone I. It was also found that the preparation of histone II was not contaminated with any component of histone I, since the electrophoretic mobility of none of the components of histone II agreed with the values obtained with histone I. The fact was also confirmed by an electrophoretic experiment on a mixture of histones I and II.

The isoelectric point of any of the components of histone II was found to be higher than pH 10.0, since none of the boundaries migrated toward the anode at this pH (see Fig. 6 and Table III).

Summary

Electrophoretic studies were made on histone I, the main fraction of calf thymus histone, at various ionic strengths, pH's and temperatures. It was found that at least three components are present in this fraction.

Histone II, the other fraction, was electrophoretically not homogeneous either. One main and three minor components were found to exist, and their mobilities differed from that of any component of histone I.

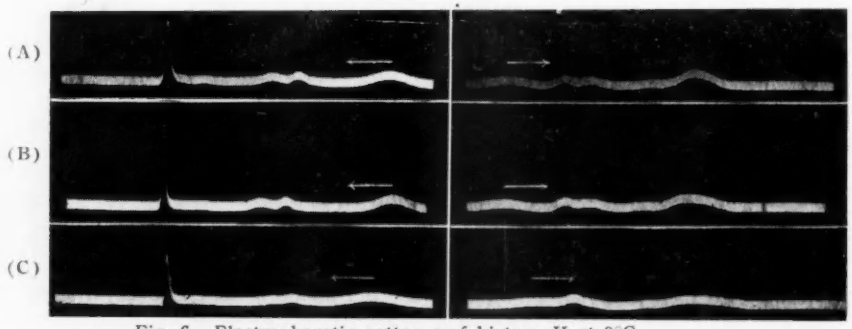


Fig. 6. Electrophoretic patterns of histone II at 0°C.
(A): In acetate buffer of pH 5.0 at ionic strength of 0.2.
(B): In acetate buffer of pH 5.0 at ionic strength of 0.1.
(C): In NH_4Cl-NH_3 buffer of pH 10.0 at ionic strength of 0.1.

TABLE III
ELECTROPHORETIC MOBILITY DATA ON HISTONE II AT 0 C*

Buffer	pH	Ionic strength		$u \times 10^5$ cm²/sec. volt			
				Comp. 1	Comp. 2	Comp. 3	Comp. 4
Acetate	5.0	0.2	Des.	**8.3**	6.5	3.9	2.7
			As.	**9.3**	7.9	4.9	3.9
Acetate	5.0	0.1	Des.	**10.1**	7.7	4.7	3.3
			As.	**12.2**	10.3	6.9	5.5
NH_4Cl-NH_3	10.0	0.1	Des.	**9.0**	6.5	3.7	2.0
			As.	**10.3**	9.1	4.9	3.9

* The values of the main component are shown in bold type.

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Further Studies on the Preparation of Calf Thymus Histone

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In a previous paper[1], it was reported that calf thymus histone, which had been extracted with sulfuric acid from the isolated nuclei and precipited by addition of ethanol at a low temperature, showed the presence of two fractions (histones I and II). In spite of their ultracentrifugal homogeneity, their electrophoretic patterns were complex and it was concluded that calf thymus histone consists of several components. However, as acids often caused irreversible modifications of many proteins, it was thought necessary to prove that no changes had occurred during the course of the extraction of histone with an acid.

In this investigation, histone was prepared by three methods without using acid, and their properties were compared with those of two preparations obtained by the acid and ethanol method[1].

Experimental and Results

(1) Preparation of Histone by Ultracentrifugation.—Calf thymus nucleohistone (TNH) is generally believed to dissociate into histone and desoxyribonucleic acid (DNA) in a concentrated saline solution[2]. In fact, when TNH was subjected to a prolonged high-speed centrifugation in concentrated saline, histone was left separate in the upper layer of the supernatant[3].

In this study, TNH* solutions both in 1 M and 2 M sodium chloride were used and different preparations of histone were obtained from the supernatants. Typical examples are as follows.

i) TNH was dissolved in 1 M sodium chloride in a concentration of about 0.4 per cent. and centrifuged at 42,040 r. p. m. in a Spinco model E ultracentrifuge using a preparative rotor B. After centrifugation for 6 hours at about 10° C, centrifuge tubes were removed as gently as possible. A sharp boundary was observed at the middle of the tube. The upper layer was withdrawn with a syringe without disturbing the boundary. It was dialyzed against distilled water and lyophilized after removing a small amount of insoluble material formed during the dialysis with a centrifuge. About 40 mg. of histone was obtained from 400 mg. of TNH.

ii) TNH solution in 2 M sodium chloride at a concentration of about 0.2 per cent. was centrifuged in the same apparatus, but a preparative rotor A was used this time and centrifuged at 50,740 r. p. m. for 5 hours. The temperature was nearly 15° C. Although no boundary was observed in this case, the upper half of the supernatant was found from the ultraviolet absorption curve to be free from nucleic acid. It was with-drawn with a syringe, dialyzed against cold distilled water and lyophilized. Twenty three mg. of histone was obtained from 160 mg. of TNH.

The preparations of histone thus obtained were essentially free from DNA and usually showed only one boundary in an ultracentrifuge. In an acetate buffer of pH 5.0 at ionic strength of 0.2, the sedimentation coefficient of the preparation obtained from the supernatant of 1 M

1) N. Ui, *Biochim. Biophys. Acta*, in press.
2) E. Chargaff, in E. Chargaff and J. N. Davidson, "The Nucleic Acids," New York, Vol. 1, p. 307 (1955).
3) A. E. Mirsky and A. W. Pollister, *J. Gen. Physiol.*, **30**, 117 (1946).

* TNH was prepared by the following method. Fresh calf thymus glands, freed from fat and extraneous tissues, were frozen over solid carbon dioxide, minced, and homogenized in an electric mixer with 0.14 M NaCl (pH 7) at a temperature below 5°C. The homogenate was then centrifuged for about ten minutes at 4,000 r. p. m. The sediment was resuspended in 0.14 M NaCl, placed in the mixer for a few minutes and centrifuged as before. The same washing was repeated three times more.

Cold 1 M NaCl was added to the sediment and the mixture was stirred overnight in a cold room. The solution was then clarified by centrifugation for 2 hours at 20,000 r. p. m. The clean but slightly opalescent supernatant was poured into six volumes of cold distilled water and a fibrous precipitate of TNH was obtained. The precipitate was washed twice with 0.14 M NaCl, and as much water as possible was pressed out of it. It was then briefly washed in water and lyophilized.

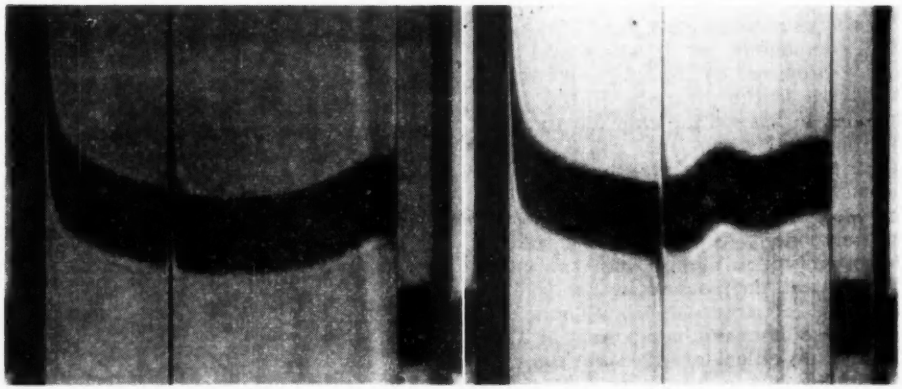
TABLE I

SEDIMENTATION COEFFICIENTS OF NUCLEOHISTONE AND SOME PREPARATIONS OF HISTONE

Sample	Solvent	Concn. (%)	$s_{20,w}$ (S) Fast	Middle	Slow
TNH	1 M NaCl	0.6	10.6	—	<1
〃	〃	0.5	11.2	—	<1
〃	2 M NaCl	0.8	4.9	3.4	<1
〃	〃	0.7	5.3	3.4	<1
Histone obtained by ultracentrifugation in 1 M NaCl	Acetate buffer pH 5.0, μ=0.2	0.7	—	—	0.7
Histone obtained by ultracentrifugation in 2 M NaCl	〃	1.0	—	1.4	?
Histone obtained by means of adsorption on Hyflo Super-Cel	〃	0.6	—	2.2	—
Histone obtained by using La salt	〃	0.7	—	1.8	?
Histone I*	〃	—**	—	2.0_3	—
Histone I*	2 M NaCl	1.0	—	3.5, 1.7	—
Histone I* once exposed to 2 M NaCl	Acetate buffer pH 5.0, μ=0.2	1.0	—	1.7	—
Histone II*	〃	—**	—	—	0.6

* These samples were prepared by the method described in a previous paper[1].

** The values were extrapolated to infinite dilution[1,4].



(A) (B)

Fig. 1. Sedimentation patterns of TNH.

(A) In 1 M NaCl after 66 min. at 59,780 r. p. m. (c=0.6%).

(B) In 1 M NaCl after 109 min. at 59,780 r. p. m. (c=0.8%).

sodium chloride solution was found to be 0.7 S and agreed with the value of histone II in previous papers[1,4] while the preparation from 2 M sodium chloride solution gave a value of 1.4 S, which was slightly lower than that of histone I (see Table I). In the case of latter preparations, the presence of a slower component was suspected from the shape of sedimentation pattern. The difference between these two kinds of preparation was also found in their solubility behavior; although the preparation from 2 M sodium chloride almost precipitated by the addition of ammonia or the same volume of 10% trichloroacetic acid, the solutions of the preparation obtained from 1 M sodium chloride remained clear on these additions. Judged from these results, the preparation from 2 M sodium chloride was thought to correspond

4) N. Ui, This Bulletin, **30**, 801 (1957).

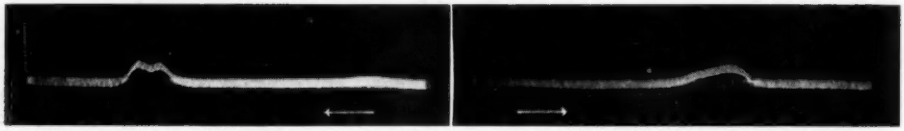


Fig. 2. Electrophoretic pattern of a histone preparation obtained by means of adsorption on Hyflo Super-Cel. Solvent: an acetate buffer of pH 5.0 at ionic strength of 0.2. Ascending pattern on left and descending pattern on right.

to a mixture of histones I and II, while the preparation from 1 M sodium chloride corresponded to histone II.

The fact that the proteins left in the upper layer of the fluid differed with the change in saline concentration, was also and more directly shown by sedimentation study of TNH. The sedimentation patterns of TNH in 1 M and 2 M sodium chloride (pH 6.3) are shown in Fig. 1. It is clear that, besides a sharp, faster boundary, one slower boundary exists in 1 M sodium chloride, and two in 2 M sodium chloride. The sedimentation coefficient of the slowest components in each case was below 1 S. A 3.4-S component was found only in the case of 2 M sodium chloride (see Table I). This component would be due to the reversible change of histone I, the 2-S component, as it was found that histone I prepared by the acid and ethanol method showed the presence of the 3.4-S component in 2 M sodium chloride and that it disappeared when the solution was brought to an ionic strength of 0.2 by dialysis.

The sedimentation coefficient of the faster component of TNH in 1 M sodium chloride was much higher than the value of the fastest component in 2 M sodium chloride or of free DNA[5]. Therefore, the faster sedimenting component of TNH in 1 M sodium chloride would not be free nucleic acid but a partially dissociated nucleohistone.

(2) Preparation of Histone by Means of Adsorption.—As histone is adsorbed on Hyflo Super-Cel from TNH solution in concentrated saline[6], an attempt was made to isolate histone by elution of the adsorbent.

TNH was extracted from the cell nuclei with 1 M sodium chloride. To the clarified extract, solid sodium chloride was added to saturation. The solution became milky and a small amount of precipitate was formed. After being kept in the cold, it was filtered with suction using Hyflo Super-Cel on a Buchner funnel. The filter cake containing histone was thus obtained*.

After being washed several times with saturated saline, the filter cake was mixed with a large volume of distilled water. Then the suspension was centrifuged and the supernatant was dialyzed against distilled water. After clarification by a centrifuge, it was lyophilized. About 400 mg. of histone was obtained from 200 g. of the glands.

The preparation of histone thus obtained contained less than 0.1 per cent. of phosphorus, so the contamination of DNA in this preparation was thought to be negligible. In an acetate buffer of pH 5.0 at ionic strength of 0.2, it showed only one sedimentation boundary and the sedimentation coefficient at a concentration of 0.6 per cent. was found to be 2.2 S. The electrophoretic pattern of this preparation in the same buffer is shown in Fig. 2. It closely resembled the pattern of histone I reported previously[1,7]. It could be precipitated by addition of ammonia or trichloroacetic acid (5 per cent.). Therefore, according to our designation, it corresponds to histone I.

Thus, only one fraction of histones, histone I, was obtained by this method and the other fraction, histone II, was not. It might be due to the failure of this histone II fraction to be retained on the filter cake under the condition mentioned above.

(3) Preparation of Histone Using Lanthanum Salt.—Lanthanum salt is known as a powerful precipitant of DNA[2]. Therefore, an attempt was made to isolate histone from TNH using lanthanum acetate.

To 100 mg. of purified TNH described before (see method (1)), 400 ml. of 2 M sodium chloride containing 3% lanthanum acetate was added and the mixture was kept in a cold room with occasional stirring. TNH swelled slowly and histone was released from it. After two days this suspension was centrifuged. The same extraction was repeated once more. The two extracts were

5) Y. Kawade, *Biochem. Biophys. Acta*, in press.
6) N. Schwander and R. Singer, *Helv. Chim. Acta*, **33**, 1521 (1950).

* This filter cake was kindly supplied by Dr. Y. Kawade.
7) N. Ui, This Bulletin, **30**, 815 (1957).

combined and, after clarification, dialyzed against distilled water and lyophilyzed. Yield, 30 mg.

When the preparation of histone obtained was subjected to ultracentrifugation in an acetate buffer of pH 5.0 at ionic strength of 0.2, only one boundary appeared. The sedimentation coefficient varied a little with the preparations i. e., 1.5—1.8 S. As the sedimentation pattern did not give a symmetrical peak, it was thought that this preparation also contained a slower component. When ammonia or the same volume of 10 per cent. trichloroacetic acid was added to its solution, a precipitate was formed, but part of the proteins remained in the solution. Therefore, this preparation was considered to be a mixture of histone I and histone II.

When TNH was extracted with the solutions of lower ionic strengths, a smaller amount of histone was extracted, presumably owing to the insufficient dissociation of TNH. In the case of 1 M sodium chloride—3% lanthanum acetate a fairly large amount of histone remained in the residue and could be extracted with 0.5 N sulfuric acid. An extract with 0.5 M sodium chloride—1.5% lanthanum did not give a precipitate in 5 per cent. trichloroacetic acid, and contained only a fraction corresponding to histone II.

Copper sulfate could be substituted for lanthanum acetate and a similar preparation was obtained.

Discussion

The three methods for the preparation of histone described above are milder than those usually adopted; neither acid, alkali nor organic solvent was used in these methods. Therefore, these preparations would be least likely to be modified during the purification. Although degradation of histone by the action of proteolytic enzyme at neutral pH was suggested by Butler et al.[8], the experiments presented here have failed to reveal it.

It was shown by the examination of these preparations that histone contained two fractions with different molecular weights. The lower molecular-weight fraction was isolated by the high-speed centrifugation experiments on TNH in 1 M sodium chloride, while the higher molecular-weight fraction was obtained by the other methods including the centrifugation in 2 M sodium chloride. Sedimentation as well as solubility studies indicated that the former corresponded to histone II described in a previous paper[1] and the latter to histone I. It was also found that the higher molecular-weight fraction was electrophoretically not homogeous.

From these results it was confirmed that the preparations of histone obtained by the standard method adopted by us[1] were not altered with acid or ethanol. The view that calf thymus histone consists of several components was also ascertained. In view of these findings, the previously reported method using acid and ethanol will be recommended, since it is easy to operate and gives a good yield.

Recently, evidences[5,9,10] were accumulated indicating that the progressive dissociation of TNH into components is induced by increasing concentrations of sodium chloride. It was also shown that dissociation was not complete in 1 M sodium chloride[5,9-11]. Failue of histone I, the higher molecular-weight fraction of histone, to be obtained by the centrifugation experiments in 1 M sodium chloride would be due to the binding of this fraction with DNA under this condition; lower molecular-weight fraction, histone II, was the only fraction present as the free protein in 1 M sodium chloride. In fact, it was shown[5] by the measurements of viscosity and sedimentation that histone I interacted with DNA in 1 M sodium chloride, while histone II did not.

Summary

Calf thymus histone was prepared as gently as possible without using acids, alkalis or organic solvents. It was clearly shown that two components with sedimentation coefficients of 2 S and 0.7 S are present.

Sedimentation, electrophoresis and solubility behaviors of these preparations were similar to those of the preparations obtained by the previously reported method using acid and ethanol, and it was confirmed that histone consists of two fractions, histones I and II. It was also ascertained that acid as well as ethanol causes no alteration of the proteins.

8) J. A. V. Butler, P. F. Davison, D. W. F. Jamse and K. V. Shooter, *Biochim. Biophys. Acta*, **13**, 224 (1954).

9) C. F. Crampton, R. Lipshitz and E. Chargaff, *J. Biol. Chem.*, **206**, 499 (1954).

10) L. B. Smillie, A. M. Marko and G. C. Butler, *Can. J. Biochem. Physiol.*, **33**, 263 (1955).

11) M. Fleming anb D. O. Jordan, *Discussions Faraday Soc.*, **No. 13**, 580 (1953).

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